

National Institute
of
Allergy and Infectious
Diseases

Annual Report of Intramural Activities

October 1, 1987-September 30, 1988

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

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For Administrative Use

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Z01-AI

00013-25 LIP
00020-13 LVD
00027-21 LMM
00030-20 LI
00035-13 LI
00036-23 LI
00043-23 LCI
00045-20 LCI
00047-19 LCI
00048-18 LCI
00057-15 LCI
00058-14 LCI
00071-17 LPB
00072-17 LPVD
00074-16 LPVD
00085-11 LPVD
00086-11 LPVD
00094-29 LPD
00097-30 LPD
00098-32 LPD
00099-18 LPD
00102-14 LPD
00108-17 LPD
00123-22 LVD
00126-15 LVD
00134-26 LMI
00135-14 LIP
00138-14 LIP
00143-19 LMI
00144-24 LMI
00145-21 LMI
00154-13 LCI
00155-13 LCI
00161-11 LPD
00162-12 LPD
00166-11 LIG
00168-11 LIG
00169-11 BRB
00170-11 LIG
00171-11 LIG
00172-10 BRB
00173-11 LIG
00180-10 LIG
00182-10 OSD
00183-10 OSD
00190-10 LMM
00193-09 LMSF
00197-09 LPD
00201-09 LPB

Z01-AI

00205-08 LIP
00208-08 LPD
00210-08 LIR
00212-08 LIR
00213-08 LIR
00216-08 LMSF
00218-07 LMM
00222-07 LMM
00224-07 LI
00226-07 LI
00234-07 LMSF
00240-07 LPD
00241-07 LPD
00242-07 LPD
00244-07 LPD
00246-06 LPD
00248-07 LPD
00249-07 LCI
00251-07 LPD
00253-07 LPD
00255-07 LPD
00256-07 LPD
00257-07 LPD
00260-07 LPVD
00262-07 LPVD
00263-07 LPVD
00264-07 LPVD
00265-07 LPVD
00266-07 LPVD
00271-07 LCI
00275-07 LCI
00277-05 LPD
00278-07 LCI
00279-07 LCI
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00284-07 LIP
00286-07 LIP
00294-07 LVD
00295-07 LVD
00296-07 LVD
00297-07 LVD
00298-07 LVD
00300-07 LMM
00301-07 LMM
00304-07 LMM
00306-07 LVD
00307-07 LVD
00308-07 LID
00309-07 LID

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1988 ANNUAL REPORT PROJECT NUMBER LISTING OF ACTIVE PROJECTS

Z01-AI

00311-07 LID
00314-07 LID
00317-07 LID
00323-07 LID
00324-07 LID
00325-07 LID
00326-07 LID
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00333-07 LID
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00341-07 LID
00342-07 LID
00343-07 LID
00345-07 LID
00346-07 LID
00347-06 LPD
00349-06 LI
00350-06 LPD
00351-06 LPD
00352-06 BRB
00353-06 LMM
00354-06 LCI
00355-06 LCI
00356-06 LCI
00358-06 LIR
00361-06 LIR
00368-06 LID
00370-06 LID
00372-06 LID
00389-05 LIG
00390-05 LIR
00394-05 LI
00397-05 LCI
00398-03 LPD
00402-05 LPB
00403-05 LI
00406-05 LID
00416-05 LVD
00417-05 OSD
00418-05 LPVD
00425-04 LI
00427-04 LI
00429-04 LCI
00430-04 LCI
00431-04 LCI
00432-04 LCI
00433-04 LMM

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00437-04 LMM
00438-04 LMM
00439-04 LPD
00440-04 LPD
00441-04 LMSF
00442-04 LMSF
00443-04 LVD
00445-04 LVD
00446-04 LID
00450-04 LID
00451-04 LID
00457-04 LID
00459-04 LID
00465-03 LIP
00466-03 LMM
00467-03 LMM
00468-03 LPVD
00469-03 LCI
00470-03 LCI
00475-03 LID
00476-03 LID
00477-03 LID
00478-03 LID
00480-03 LPB
00481-03 LCI
00482-03 LCI
00483-03 LPD
00484-02 LIP
00485-02 LCMI
00486-02 LCMI
00487-02 LPD
00488-02 LPB
00489-02 LPB
00490-02 LPB
00491-02 LPB
00492-02 LPB
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00494-02 LPD
00495-02 LCI
00496-02 LID
00497-02 LID
00498-02 LID
00499-02 LID
00500-02 LID
00501-02 LID
00502-02 LID
00504-02 LID
00507-02 LID
00510-02 LID

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1988 ANNUAL REPORT PROJECT NUMBER LISTING OF ACTIVE PROJECTS

Z01-AI

00512-01 LPD
00513-01 LCI
00514-01 LCI
00515-01 LMSF
00516-01 LMSF
00517-01 LPD
00518-01 LMSF
00519-01 LMSF
00520-01 LI
00521-01 LCI
00522-01 BRB
00523-01 BRB
00524-01 LPVD
00525-01 LIG
00526-01 LMM
00527-01 LMM
00528-01 LMM
00529-01 LCMI
00530-01 LID
00531-01 LID
00532-01 LID
00533-01 LID
00534-01 LID
00535-01 LPD
00536-01 LPD
00537-01 LIR
00538-01 LVD
00539-01 LVD
00540-01 LVD
00541-01 LVD
00542-01 LVD
00543-01 BRB

OFFICE OF THE SCIENTIFIC DIRECTOR, NIAID
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Summary of Program
Laboratory and Clinical Research, NIAID
October 1, 1987 - September 30, 1988

The NIAID Intramural Research Program (IRP) consists of 13 laboratories, a Biological Resources Branch, Administrative Management Branch and Animal Care Branch. Three laboratories are located in Hamilton, Montana at the Rocky Mountain Laboratories. The remaining facilities are concentrated at the NIH Bethesda Campus with auxiliary facilities at Frederick (Ft. Detrick) and Rockville, MD. The Office of the Director, IRP, (also called Scientific Director) is responsible for the management of the entire Intramural Program. In FY'88 the NIAID IRP had an allocation of \$77,604,000; about 31% of the budget for salaries, 24% for the NIH management fund and the remainder for laboratory expenses, costs of laboratory support contracts and travel support. In FY'88 there were 768 persons working within the NIAID IRP with 86 tenured scientists, 167 nontenured scientists, 179 guest workers and 336 nonscientists.

This past year several significant changes occurred within the NIAID IRP. The Laboratory of Microbial Immunity was closed. Dr. Claude Garon was appointed Chief of the Laboratory of Pathobiology at the Rocky Mountain Laboratories. In FY'88 NIAID acquired new facilities as part of the expansion of the IRP AIDS program. A building was renovated in Frederick, MD to house contract support of the IRP AIDS program and 25,000 sq. ft. of new laboratory space was added to our AIDS facility in Rockville, MD. In September, 1988 NIAID moved from Building 5 on the Bethesda campus to a freshly renovated building (building 4). In order to facilitate interaction among NIAID IRP scientists located in widely dispersed geographic regions, a local area network linking all laboratories was developed and should be fully operational by the end of 1988.

Numerous accomplishments were made by NIAID IRP scientists this past year. In the area of AIDS research important animal models were developed using transgenic mice and rabbits. In addition, studies of simian and feline immunodeficiency viruses were initiated as models of the human immunodeficiency virus (HIV). In other studies of AIDS human monocytes were shown to be a reservoir for HIV, an immunoassay to detect HIV in as few as one in a million infected cells was developed, partial reconstitution of the immunologic defects in certain AIDS patients was accomplished by bone marrow transplantation, the first vaccine trial for HIV using a recombinant GP160 protein was initiated, a high rate of HIV among patients attending a sexually transmitted disease clinic in Baltimore, MD was demonstrated, the binding site of HIV to cells was shown to be the CD4 receptor, and a chimeric protein containing CD4 and active portions of pseudomonas exotoxin was shown to kill cells infected with HIV.

Over two-thirds of the NIAID IRP research effort was directed at nonAIDS activities. In the area of vaccine development there were numerous important advances. These included increasing the safety of live recombinant vaccinia virus vaccines originally developed by NIAID IRP investigators, the successful immunization of mice with a vaccinia-dengue or baculovirus-dengue recombinant viruses, the isolation of hepatitis A from humans and the successful immunization of chimpanzees using an attenuated virus, completion of field trials evaluating a rotavirus vaccine and demonstration of a need for protection against four virus strains resulted in making reassortment (hybrid) viruses which are now in clinical trials. A respiratory syncytial virus (RSV)-vaccinia recombinant expressing the attachment and fusion protein of RSV has been made and shown to protect monkeys against infection. In other studies the immunogenic region of Chlamydia trachomatis major outer membrane has been identified, cloned and inserted into salmonella and polio vectors which will be tested as potential vaccines, the protein that is the target of a passively-protective antibody against Rickettsia rickettsii has been cloned and is under evaluation as a vaccine, and efforts to develop a malaria vaccine resulted in cloning the gene for one of the antigens important in the sexual stages of parasite development. As part of

NIAID's effort to develop a "clean" vaccine against Bordetella pertussis the gene encoding the protective toxin has been altered to produce a molecule with no biologic activity but with immunogenic capability.

Investigation of basic and clinical immunology has led to early studies of immune modulation in patients with immune deficiencies. Defective hydrogen peroxide production by phagocytes of patients with chronic granulomatous disease of childhood has been corrected in vitro and in vivo with interferon-gamma and clinical trials will begin shortly. NIAID IRP investigators demonstrated that production of IgE antibodies important in allergies are controlled by interleukin-4 and by interferon-gamma. Important immunoregulatory functions have been attributed to tumor necrosis factor-alpha. In other studies important functional abnormalities of high molecular weight B cell growth factor receptor in patients with common variable immunodeficiency have been defined. Many other outstanding accomplishments by NIAID IRP investigators in FY'88 in the host defenses and allergic diseases have been made and are presented in the annual report from each laboratory.

Equal Employment Opportunity and Affirmative Action Programs have been given a high level of attention. Our annual Introduction to Biomedical Research Program for College Minority Students from across the nation was held in February. This year we had an active summer program for high school, college and medical students with 45 students working in NIAID laboratories; 49% of students in the summer program were minorities.

December 7-9, 1987 the NIAID Board of Scientific Counselors, under the leadership of Dr. Robert B. Couch, reviewed the Laboratory of Infectious Diseases (Dr. Robert Chanock, Laboratory Chief), and the Department of Transfusion Medicine, Clinical Center, NIH (Dr. Harvey Alter, Head). May 23-25, 1988 the Board of Scientific Counselors reviewed the Laboratory of Parasitic Diseases (Dr. Franklin Neva, Laboratory Chief), and the Hospital Epidemiology Service, Office of the Director, Clinical Center, NIH (Dr. David Henderson, Chief). Each of these laboratories received laudatory reviews. This year Drs. Priscilla Schaffer and Stanley Falkow completed their terms on the Board and Drs. Zanvil Cohn and Rochelle Hirschhorn joined the Board.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00417-05 OSD
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Systematics and Vector Relationships of Ticks (Ixodoidea)</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: James E. Keirans Research Entomologist OSD, NIAID OTHERS: Richard G. Robbins Museum Specialist OSD, NIAID		
COOPERATING UNITS (if any) Dr. C.E. Yunker, Heartwater Project, Harare, Zimbabwe; Drs. D. Sonenshine and P. Homsher, Old Dominion University; Dr. J. B. Walker, Onderstepoort, R.S.A.; Dr. R.G. Endris, Plum Island Animal Disease Laboratory, USDA.		
LAB/BRANCH Office of the Director of Intramural Research		
SECTION Entomology Department, Museum Support Center, Smithsonian, Wash., D.C.		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The activities of this research project currently comprise three main functions: (1) Identification of ticks received from various individuals, universities and governmental agencies throughout the world. This is the only laboratory in the world capable of performing this function; (2) Systematic investigations of the Ixodoidea including taxonomy, classification, and life histories; and (3) entry, retrieval and manipulation of our tick data base on the Smithsonian computer system to provide researchers with the latest updated information on any tick species.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical & Genetic Mechanisms of Obligate Intracellular Parasitism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

J. C. Williams, Ph.D., Sr. Scientist, NIAID/OSD, Chief, Rickettsial Diseases Laboratory, USAMRIID

T. A. Hoover, Ph.D., National Research Council Fellow

N. Bhatnagar, Ph.D., National Research Council Fellow.

H. A. Thompson, Ph.D. Visiting Scientist and Associate Professor, Dept. Microbiol and Immunol, West Virginia University Medical Center, Morgantown, WV

S-Y. Chen, M.S., Pre-doctoral candidate

COOPERATING UNITS (if any)

M. H. Vodkin, Ph.D., University of Illinois, Urbana, IL

LAB/BRANCH

Office of the Director of Intramural Research Program, NIAID, Bethesda, MD

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD and Bldg. 550, Ft. Detrick, Frederick, MD 21701

TOTAL MAN-YEARS

4.5

PROFESSIONAL

3.5

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of this project were the characterization of two recently cloned genes and the expression of a previously cloned gene under the control of a heat shock promoter (hsp) from Coxiella burnetii. A. Gene cloning. Genomic DNA potentially encoding aspartate transcarbamoylase (ATCase, pyrB) was cloned into the plasmid pEMBL8+. A 7 kb fragment was selected for its ability to complement a pyrB-E. coli auxotroph. The putative C. burnetii pyrB gene was shown to express an active ATCase using cell-free extracts of the recombinant E. coli. DNA sequence analysis of a 2.2 kb subclone revealed a product of approximate size and composition in common with the pyrB gene of E. coli. B. Surface antigen. The product of a 2.5 kb fragment from the same EcoRI genomic library was selected by monoclonal antibodies raised against a 29.5 kd immunodominant surface protein. DNA sequence analysis revealed an open reading frame encoding a 30 kd lipoprotein. The amino acid composition of the purified 29.5 kd surface protein conflicts with the deduced amino acid composition of the cloned lipoprotein gene product detected by monoclonal antibodies. C. HSP. A recently cloned operon, htpAB, under the control of an hsp from C. burnetii expressed 62 and 14 kd gene products at 42°C in the recombinant E. coli. A similar study in C. burnetii indicated that both of these and at least 5 more genes were maximally expressed at 42°C. In addition a cold-shock gene product was only expressed at 23°C. D. Significance. The C. burnetii pyrB gene resembles structurally the gene of E. coli, but appears to be regulated differently. An immunodominant 29.5 kd surface protein may share a common epitope with other similarly sized lipoproteins. The induction of gene expression during stress responses under the control of hsp may contribute to the survival of C. burnetii in the phagolysosome.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunologic Properties of Coxiella burnetii (Q fever) Vaccine

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J. C. Williams, Ph.D., Sr. Scientist, NIAID/OSD, Chief, Rickettsial Diseases Laboratory, USAMRIID

G. H. Scott, Ph.D., USAMRIID

D. M. Waag, Ph.D., USAMRIID

C. R. Bolt, M.S., USAMRIID

T. F. McCaul, Ph.D., Sr. Fellow, National Research Council Fellow

COOPERATING UNITS (if any)

LAB/BRANCH

Office of the Director of Intramural Research Program, NIAID, Bethesda, MD

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:

5.0

PROFESSIONAL:

4.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of this project were the characterization of immunogenic and pathogenic fractions of Coxiella burnetii based on humoral and cellular mechanisms of immunity. A. Lipopolysaccharide (LPS) vaccine. In A/J mice the phase I LPS was nontoxic at a dose of 100 µg and it stimulated significant antibody titers and lymphocyte responses against LPS, phase I and II cells. A single injection of 2.5 µg gave complete protection against virulent aerosol challenge without a detectable pre-challenge immune response. Western blot analysis of the antibody activity indicated that peptides which co-purified with the LPS induced anti-protein reactions. B. Biological response modification (BRM). Both phase I whole cells and CMR are effective inducers of tumor necrosis factor and interferon. Immunization of C57BL/10 J mice with phase I whole cells induced time and dose dependent immunosuppression without simultaneous increases in T suppressor cells in the spleen, but with the appearance of a new population of immunoglobulin bearing thymocytes. C. Antigenic shift. Morphologic variants of the C. burnetii developmental cycle undergo stage specific antigenic changes of their cell surface detected by immunogold and monoclonal antibody labels. The endospore was not recognized by the immune response. E. Significance. The phase I LPS may be an effective vaccine performing both as an adjuvant and peptide carrier. The BRM of the humoral and cellular immune responses by several fractions enhances specific and non-specific immunity. Stage specific antigenic changes of morphologic variant surfaces may be a mechanism of evading the immune response and may lead to the development of chronic infections and reactivation of Q fever.

ANIMAL CARE BRANCH

1988 ANNUAL REPORT

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ANIMAL CARE BRANCH

1988 Annual Report

Summary

Much of the current research in the understanding and treatment of allergies and infectious diseases requires the use of laboratory animals. The care and treatment of these animals can have a profound affect on the results of experimental procedures. An animal's environmental conditions, its daily care, the presence or absence of disease-causing microorganisms, the amount of pain or distress that results from experimental manipulations all may affect the validity of research data. The NIAID intramural scientists today are very sensitive to these factors and their ethical responsibility for ensuring that animals used in research receive high quality animal care. All NIAID scientists conducting research that involves animals are required to attend the NIH course on "Using Animals in Intramural Research: Guidelines for Investigators." This course offers not only a broad perspective on current animal issues but also provides specific information on actual techniques and procedures.

It is the responsibility of the NIAID Animal Care Branch to provide the daily care to all animals maintained within the NIAID animal facilities, to oversee NIAID's intramural contracts involving animals, and to assist NIAID scientists with animal-related problems. The NIAID maintains four animal facilities at the NIH campus in Bethesda, Maryland, two animal facilities at the Frederick Cancer Research Facility in Frederick, Maryland, and four animal facilities at its Rocky Mountain Laboratories at Hamilton, Montana. Approximately 54,000 square feet of space (22,000 sq. ft. at the NIH in Bethesda, MD; 2,500 sq. ft. at the FCRF in Frederick, MD; and 29,000 sq. ft. at the RML in Hamilton, MT), excluding pasture land, are devoted to the NIAID Animal Care Program.

The NIAID's Animal Care Branch provides guidance to the Institute's intramural scientists requiring animals in their research projects. This guidance includes assistance in the selection and proper administration of anesthetics and analgesics, the purchase of animals, the diagnosis, treatment, and control of infectious agents, and the performance of various technical procedures in laboratory animals. The Branch operates one of the only containment facilities in the country for infectious disease studies involving animals. This fully-accredited animal facility is located in Frederick, Maryland, and can maintain animals on studies requiring Biosafety Level 3 and/or 4 safeguards. It is available to any scientist with an approved protocol that requires maximum isolation for work with an infectious agent. The Branch maintains production colonies of over sixty different strains of mice, hamsters, cotton rats, and rabbits for NIAID investigators within its or contracts' animal facilities. Many of these animals are unavailable anywhere else in the world or are available only after long delays.

The NIAID Intramural Research Program is fully committed to meeting all Federal rules and regulations pertaining to the use of animals in biomedical research. In December, 1987, the NIAID's Animal Care Branch relocated all the animals maintained in its central animal holding facility in Building 7 so that this facility could be brought into full compliance with Federal construction guidelines for animal facilities and be enlarged to provide quarantine and isolation facilities, laboratory space for animal procedures, additional animal holding space, and space for the storage of supplies and equipment. This project is scheduled to be completed in September, 1987.

In addition, the NIAID is constructing a new primate and rodent facility in Building 4 that will be completed in the fall of 1988. This facility will also fully meet the construction guidelines mandated by the Public Health Service policy on the Humane Care and Use of Laboratory Animals and will provide procedure space, separate from the animal rooms, in which investigators can perform experimental manipulations on animals.

A major goal of the NIAID Animal Care Branch during FY88 is to identify space for, and hire additional personnel to manage, the increased clerical and administrative responsibilities the Branch has had to assume to comply with the NIH policy on Animal Care and Use in the Intramural Program and the Public Health Service policy on the Humane Care and Use of Laboratory Animals. The Branch did hire a second veterinarian in FY87 to improve veterinary services within the NIAID intramural program.

ADMINISTRATIVE MANAGEMENT BRANCH

ANNUAL REPORT FY 1988

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ADMINISTRATIVE MANAGEMENT BRANCH
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASE

October 1, 1987 to September 30, 1988

Introduction

The recent impetus for combating AIDS and the resulting staff and budget increases have multiplied the amount of administrative support needed in the Intramural Research Program. With little corresponding increase in the Administrative Management Branch's staff, we have been able to keep pace with the challenge and to provide the highest quality administrative support and advisory services to the IRP. The AMB has completed a very rigorous fiscal year filled with numerous accomplishments--all instrumental in improving, maintaining, and expanding administrative support to the scientific staff. The AMB continued to be involved in budget administration, contracts, renovations, space management, automation and procurement. The high quality of our work product has been verified through time and attendance, financial and procurement audits. The following will provide a brief exposition of our activities and accomplishments throughout Fiscal Year 1988.

Budget and Financial Management

In FY'88 IRP's budget allocation increased by 7.6% over FY'87 to a total of \$77.7 million. The Administrative Management Branch made numerous advances in administering and managing the IRP budget. Improved methods have been devised for tracking and reporting the budgets and financial status of the IRP's laboratories, branches, and offices. The Acquisition Management and Budget Information System (AMBIS) was developed and implemented. It provides for sharing of procurement and budget data between the laboratory and the administrative staff. AMBIS allows laboratory personnel to enter Requisition Worksheet information into the NIAID Local Area Network (LAN) and to transmit that worksheet directly through the Network to the Purchasing Agent. Vendor information is maintained in computer files and is readily available to laboratory personnel when entering worksheet information. Most importantly, a current budget status for all the labs is available at any time to both the administrative staff and to authorized lab personnel. Building 10 administrative staff and Laboratory of Clinical Immunology are currently using the new system. The remainder of Building 10 labs are scheduled to begin using AMBIS on September 1, 1988 and all IRP as well as NIAID Extramural Program will be utilizing the new system by December 1988. Monthly Status of Funds reports have been established using Lotus 1-2-3 computer software. These reports give detailed information regarding supplies and services, equipment and travel budgets for each laboratory, as well as financial projections based on current spending levels. Accessing the Wylbur computer system, the AMB has also been able to provide weekly reports on obligations and commitments useful to the administrative staff and labs in managing their budgets. In addition, DFM weekly reports and SSF monthly reports are also carefully monitored and analyzed to examine spending in specific areas such as animals, photography, shipping, etc.

Renovations and Space Relocations

During this year the AMB has been actively involved in managing and overseeing numerous lab and office space renovations. Many of these renovations have been precipitated by the need for additional and/or more functional space to conduct AIDS research. Major renovations have occurred in all Buildings occupied by IRP staff, from completely renovated and modernized Cold Room space to lab modules and offices. In addition, AMB has successfully coordinated and overseen the completion of major renovations such as the FCRF Building 550/469, Twinbrook II (Phase I renovation) and Building 4. Most IRP staff in Building 5 will be moving to the newly renovated space in Building 4 beginning September 1, 1988. The administrative staff in Building 7 are in the process of relocating to newly renovated space on the second floor of this building. And finally, to keep track of all IRP space and staff locations, the Branch has developed a computer-generated Space Report. This tool has made the management of space a less cumbersome task.

Procurement/Contracts

The demand for purchasing supplies, services and equipment in order to carry out this vital research has also significantly increased. However, staffing levels for IRP Purchasing Agents have remained constant except for the addition of 1 Purchasing Agent at the Twinbrook facility. AMB's Purchasing Agents have continued to meet the challenge of increased staff demands with a phenomenal level of efficiency and accuracy. In fact, procurement practices have come under close scrutiny this fiscal year and we have been commended for our efficient way of performing procurement functions. AMB's work in the procurement area has become a standard of excellence throughout the NIH. Earlier this year, NIH contracted with the Logistics Management Institute (LMI), to conduct a review and evaluation of its procurement practices and a final report was issued in April 1988. Although the report highlighted areas of concern at the NIH, we received high valued praises in performing our procurement functions. Other administrative accomplishments in the procurement area include: development of a monthly newsletter entitled "Procurement Tips" to be used by all NIAID administrative staff; establishment of a Requisition Worksheet, NIH Form 1861-3 to facilitate lab and administrative staff procurement efforts and to enable the administrative staff to more readily comply with federal procurement regulations; development of written procedures and procurement guidelines for use by laboratory personnel; creation of an automated file listing utilizing the LAN system for approximately 500 vendors; and improvement of filing systems in all administrative offices. We have also taken steps to formalize our contract review process for both procurement and research and development contracts. This has included the appointment of an Administrative Officer to oversee contracts and monitor expenditures, a revised standardized concept review form for use by our Board of Scientific Counselors and an annual contract review for the Director, IRP to support the scientific evaluation and funding of ongoing and new projects. Finally, in an effort to more effectively manage contractor operations at the FCRF, the administrative staff obtained approval to consolidate all of the IRP's scientific programs concerning vaccine development and treatment under one project. This consolidation produced positive results for budget tracking, space management and day-to-day operations.

Automation

The Local Area Network (LAN) has been installed in Buildings 10, 7, 5, Twinbrook, and FCRF. Most obsolete word processing equipment such as the IBM 5520 has been replaced by PCs. These machines are supporting a high level of word processing and enabling the user to accomplish many other tasks, such as network mail, telecommunications and database-type applications. The LAN has resulted in a cost savings since various software programs and equipment can be shared thereby eliminating the need for individual purchases. The AMB has been instrumental in ensuring that all IRP staff are trained on the new system. In addition, procedures were implemented to assist the labs in ordering ADP equipment and to insure that they are requesting compatible equipment with the LAN. In order to fulfill the automation needs of the IRP, a contract computer specialist was hired by the Institute this fiscal year. Computer support is also augmented by a computer specialist from DCRT assigned to IRP. Projects completed along with AMB assistance include an Employee Performance Management System which is a database-oriented, menu-driven program, written specifically for the Institute and used primarily by employees and supervisors to implement a more flexible and realistic approach to the creation of appropriate EPMS forms; the aforementioned Space Report; the AMBIS System that allows scientists in the labs to expedite ordering, reduce paperwork and permit Purchasing Agents to track orders and maintain lab budgets more efficiently; DELPRO installation on the Network which has enabled the user to interact with the IMS from any computer on the Network and has decreased the cost for AT&T rentals for DELPRO. The installation of DELPRO on the network will also allow for an efficient transition of NIH central services on-line and easy access for NIAID staff.

Personnel

Positions ceilings for AMB remained unchanged this fiscal year with the exception of an additional FTE. Staffing levels were fairly stable except some turnover in the Purchasing Agent positions. At the end of the fiscal year, however, all administrative offices are fully staffed with 25 FTEs encumbered plus three Stay-in-School employees. Plans are underway for establishing to a Lead Purchasing Agent, GS-8 and for reorganizing the AMB into two sections under the Office of the Chief, AMB. In order to increase the flow of communication and to provide as much administrative support as possible, Administrative Officers meet regularly with the lab chiefs in their respective areas. The Chief, AMB's selection to serve on various NIH and NIAID committees escalated this fiscal year and she has expended considerable efforts assisting in developing and implementing NIH's Patent Policy. She is chairing the Intramural Administrative Officers Committee and provides administrative expertise as a member of the following committees: Patent Policy Training Subcommittee, Service and Supply Fund Activity, NIH-ADAMHA Industry Forum, DES--Intramural Administrative Officers Working Group, Patent Policy BID Liaison, NIAID Procurement Oversight Official, Clinical Review Subcommittee, and the Promotion Advisory Committee.

BIOLOGICAL RESOURCES BRANCH
1988 Annual Report
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ANNUAL REPORT
BIOLOGICAL RESOURCES BRANCH
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
OCTOBER 1, 1987 TO SEPTEMBER 30, 1988

RESEARCH PROGRESS

The Biological Resources Branch investigates the structure and function of the genes and molecules involved in the immune response, the molecular basis for antigen presentation and recognition, and hemopoietic cell differentiation. In addition to this fundamental immunological research, the Branch provides protein sequencing, peptide synthesis and flow cytometry expertise and services for the NIAID.

STUDIES OF THE MAJOR HISTOCOMPATIBILITY COMPLEX

Major histocompatibility complex (MHC) encoded class I molecules are integrally involved in the presentation of viral antigens, in the form of processed peptides, to cytotoxic T lymphocytes (CTL). Studies on the molecular basis of viral antigen processing and the nature of the interaction of processed antigen (peptides) with class I molecules, as well as factors controlling the expression of class I molecules have been emphasized in this research.

Presentation of Viral Peptide Antigens by Class I Molecules. Previous studies have suggested that MHC class I molecules bind and present peptides to CTL, and that the peptides bind to two α helices in the class I molecule consisting of residues 50-84 and 138-180. For the M1 peptide of the influenza virus matrix protein, it was shown that the amino acid substitutions at positions 152 and 156 of HLA-A2.1 in the second α helix are important for recognition by influenza virus restricted CTL. However, it was shown that the failure to recognize complexes between the peptide and these mutant HLA-A2.1 molecules was due to T-cell receptor specificity and not the failure of the HLA-A2.1 molecules to bind peptide (Coligan, Maloy). For CTL recognition of HLA-A3 class I molecules the nature of the amino acid difference at position 152 was shown to be critical for distinguishing HLA-A3.1 and HLA-A3.2 molecules either by allo-immune CTL or virally restricted CTL. Different viruses and target cells did not alter this recognition pattern. Thus, both of these studies emphasize that the region between residues 150 and 160 of class I molecules as being critical for antigen presentation to cytotoxic T lymphocytes (Coligan).

Identification of Viral Peptides Presented by Class I Molecules. To define the parameters of interaction of viral peptide antigens with MHC class I molecules, it was necessary to define peptide antigens recognized in the context of H-2 class I molecules available in soluble form i.e. H-2K^D and H-2D^d. The influenza A virus basic polymerase 2 (PB2) was found to be recognized by H-2D^d restricted influenza virus specific CTL, and of the thirty one peptides synthesized, three peptides from PB2 were found to be recognized by the virus specific CTL. These CTL epitopes have low amphipathicity values, but fit the Rothbard algorithm for T cell epitopes. The peptides that have been identified have been radiolabeled and the class I molecule binding studies have begun. In addition, four epitopes recognized by SV40 specific, H-2D^D restricted CTL have been identified and studies on H-2K^D epitopes are in progress (Anderson, Maloy).

Minimal Antigenic Requirements for the Generation of a CTL Response. It has been previously shown that MHC restricted cytotoxic T lymphocytes recognize foreign antigen in the form of short peptides. In defining the minimal requirements for the induction of virus specific CTL, a peptide containing epitopes from the influenza type A matrix (M1) protein recognized by both human CTL and T helper cells has been shown to sensitize target cells for recognition by influenza A virus specific CTL. This peptide was also shown to induce cytolytic effector cells from human peripheral blood lymphocytes (PBL) when cultured with the PBL even in the absence of exogenous growth factors. These data indicate that virus specific CTL can be stimulated *in vitro* with the CTL epitope and the lymphokines secreted by the T helper cells induced by the T helper cell epitope. This information will be important for the design of synthetic peptide based vaccines for the production of CTL mediated immunity (Anderson).

Multiple Class I Molecules from a Single Gene. The goal of these studies is to gain an understanding of the regulation of expression, and the functional and antigenic properties of these molecules. It has been shown that multiple class I molecules can be generated from single genes by alternative splicing of pre-mRNAs. Particular alternatively spliced gene products affecting the C-terminus have been shown to distinguish H-2K molecules from H-2D molecules (Coligan, Maloy). For H-2K molecules, the splicing pattern favoring the use of a long exon 8 could be correlated with a potential lariat branch point adenosine 28 bp upstream of the splice junction. Elimination of this adenosine altered the pattern of pre-mRNA splicing in H-2K molecules resulting in a predominance of short exon 8 encoded sequences. The splicing pattern of H-2D region molecules could not be correlated with this base alone (Handy).

MOLECULAR STUDIES ON T CELL RECEPTORS

The $\alpha\beta$ T cell Receptor. Multiple molecules exist on the surface of lymphocytes that are important for the development, activation and regulation of the immune response. Of prime importance in this regard is the clonotypic $\alpha\beta$ T cell receptor that is responsible for antigen recognition. Recent studies have emphasized factors regulating the biosynthesis and expression of this receptor.

The biosynthesis, processing, and assembly of the T cell receptor α - and β -chains with each other and with the CD3 complex were investigated on both cell surface positive (TCR⁺CD3⁺) and negative (TCR⁻CD3⁻) cell lines. The results indicate that 1) in the cell surface TCR⁺CD3⁺ cell lines (MOLT 3, CCRF-CEM), TCR- β , but not α -chains are present intracellularly. TCR- β -CD3 complexes are readily detectable in these cell lines, but no evidence for final processing or cell surface expression of such incomplete TCR-CD3 complexes is observed. 2) In the cell surface TCR⁺CD3⁺ cell line HPB-ALL, both α - and β -chains are present intracellularly. Whereas non-glycosylated forms of TCR- β chain can be detected, only more mature forms of TCR α -chains are detected indicating that the α -chains are more rapidly glycosylated than the β -chains. 3) The large majority of the intracellular α - and β -chains are not disulfide linked and a small fraction of these are associated with CD3. 4) Only small amounts of the total intracellular TCR chains are found as CD3-associated disulfide-linked $\alpha\beta$ -heterodimers. 5) Final processing of TCR chains for cell surface expression takes place after formation of these TCR- $\alpha\beta$ -CD3 complexes. Thus, both the TCR α - and β -chains are over-produced and only relatively small amounts of these chains form CD3-associated heterodimers that are processed for cell surface expression. Analogous results were obtained with a non-leukemic CTL clone.

Based on these observations, a model for the biosynthesis and assembly of the TCR-CD3 complex was proposed (Koning).

The $\gamma\delta$ T Cell Receptor. The antigen receptor (TCR) on helper and cytotoxic T lymphocytes consists of an $\alpha\beta$ chain heterodimer that is associated with the CD3 complex on the cell surface. Recently, a second CD3-associated heterodimer, composed of a γ gene product and a δ gene product, has been identified on a subset of thymocytes. Because β and γ chain mRNA, in contrast to α chain mRNA transcripts, are abundant in immature murine thymocytes, it was hypothesized that such thymocytes could express $\beta\gamma$ receptors that are replaced by $\alpha\beta$ receptors after translation of α chain mRNA. However, no evidence existed that β and γ protein can be synthesized by a single cell. It was shown that the human T cell line PEER, which expresses a CD3 associated TCR γ chain on the cell surface, synthesizes a 38 kD β chain product that associates intracellularly with CD3. However, this CD3-TCR β complex is not found on the cell surface, presumably because no α chain protein can be synthesized. It was concluded that β and γ proteins can be produced within the same cell and that they can independently form CD3-TCR β and CD3-TCR γ chain complexes. However, CD3-TCR β γ complexes apparently do not form (Koning, Coligan).

Analyses of the $\gamma\delta$ TCR revealed significant clonal diversity in expression indicating that the $\gamma\delta$ TCR has the potential to recognize a variety of ligands (Koning). Furthermore, the activation properties of cells bearing $\gamma\delta$ TCRs was found to be very similar to those bearing $\alpha\beta$ TCRs in terms of CD3 components and responsiveness to various activation signals such as anti-Thy-1, anti-CD3, PMA and ConA. The majority of $\gamma\delta$ expressing cells present in the periphery were found to be thymic dependent with a small percentage being thymic independent. Multiple forms of $\gamma\delta$ TCRs were found to be present in the periphery including those containing several types of C γ chains. In addition, whereas CD3⁺, CD4⁻, CD8⁻, $\alpha\beta$ TCR⁺ cells have been found in the thymus, none could be detected in the periphery (Coligan).

SYNTHESIS OF PEPTIDE ANTIGENS

The major objective of this project is the chemical synthesis of peptides that correspond to portions of a native protein. These peptides are then used to mimic the reactivity of the native protein or are used to make anti-peptide sera that will crossreact with the native protein. The peptides are synthesized for many different projects, each with its own specific objective, however in general the anti-peptide sera are used as probes for a native protein or for a specific region of a protein, while the peptides that are not used to make antisera are used to identify and mimic epitopes recognized by antibody, T helper, or cytotoxic T lymphocytes (CTL). During the past year, 450 peptides have been synthesized for 50 investigators in the NIAID (Maloy). Synthetic peptides and anti-peptide sera are being used in the manner just described to investigate many different proteins, that include products of the human and murine MHC; human and murine T cell receptor proteins; viral proteins from HIV-1, hepatitis A and B, rota virus, dengue virus, vaccinia virus and various strains of influenza virus; parasite proteins from malaria, schistosoma and the trypanosoma; lymphokines IL-1 and IL-5; lymphocyte antigens Ly 5 and CD4.

PROTEIN SEQUENCE ANALYSES

The facility has analyzed over 220 samples during the past nine months and projects that it will analyze about 300 samples this fiscal year from the following sources:

NIAID	Sample No.
LPD	50
LI	15
LVD	30
LMM	10
LIR	15
LCI	5
BRB	20
LID	<u>20</u>
Sub total	165
NCI	15
NINCDS	2
NIDDKD	3
Extramural	<u>25</u>
Total	220

In addition to sequence analyses, in the case of several malaria proteins and a schistosome protein, the facility has purified tryptic peptides for sequence analysis by reverse phase microbore HPLC separation. Several studies in which data provided by the facility have had a major impact are listed below:

(1) the characterization and identification of the putative *gag-pol* protease and *gag* encoded gene products of human immunodeficiency virus (HIV) (Laboratory of Molecular Microbiology); (2) the characterization of human pro-gastrin-releasing peptides expressed in baculovirus (Navy Medical Oncology Branch, NCI); (3) the identification of previously unidentified vaccinia virus encoded proteins (Laboratory of Viral Diseases); (4) the characterization of high-efficiency purified interleukin 4 (Laboratory of Immunology); (5) the structure of T-cell activating protein (TAP) and related Ly-6 encoded molecules (Harvard Medical School); (6) the identification of a chimeric MHC class II/class I protein expressed on the cell surface (Laboratory of Immunology); (7) protein sequence determinations which allowed the development of oligonucleotide probes for the cloning of PFs25 that is expressed on zygotes and ookinetes of *P. falciparum* (Laboratory of Parasitic Diseases); (8) the identification of hamster MHC class I antigens (Laboratory of Immunopathology); (9) analysis of the BCDF (II-6) receptor (Laboratory of Immunoregulation); (10) identification of Dengue virus pre-matrix structural protein (Laboratory of Infectious Disease).

FLOW CYTOMETRY ANALYSES

The Flow Cytometry Facility (Holmes) has undergone considerable change in the last fiscal year. It has incorporated two new flow cytometers, hired and trained 3 new

employees and instituted a new computer support contract for the section. All 12 IRP Laboratories have utilized the facility, using approximately 2158 hours of instrument time for single- and multi-parameter analysis and sorting. In addition, the user-operated instruments are generating an average of 30 files/day (approx. 1-2 hrs/day).

The Flow Cytometry Facility has provided support to all 12 laboratories in the IRP and many non-NIAID labs, and therefore has impacted on a wide range of research projects. These include: 1) immunology: T and B cell ontogeny, effects of interleukins/biological response modifiers on lymphoid cells, B and T cell differentiation and physiology 2) virology: AIDS related research, murine leukemia viruses, murine immunodeficiency retrovirus, vaccinia virus; 3) parasitology: defense mechanisms to parasite invasion; 4) molecular biology: study of a variety of genes transfected into cultured cells.

As the reliability of the instrumentation/personnel has become established in the facility, the frequency of repeat experiments has diminished while at the same time there has been an increase in the number of new users and projects. We expect that the level of use will increase further, particularly for sorting and multiparameter analysis. This increase was anticipated and influenced the decision to purchase the FACStar Plus and analyzers.

BIOLOGICAL RESOURCES BRANCH

Annual Report

October 1, 1987 to September 30, 1988

ADMINISTRATIVE REPORT

The period covered by this annual report marks the inaugural year of the Biological Resources Branch. There are four Sections within the Branch; most of which are primarily involved with providing technological resources to the NIAID. Despite difficulties in keeping personnel trained in highly sophisticated techniques from being hired by other organizations, all of the service facilities have at least doubled their output within the last year. This was mainly due to reorganization, new instrumentation and/or the hiring of highly skilled personnel. A major change in the Protein Sequencing Facility was the departure of Mr. Michael Raum, an individual with 8 years of protein sequence experience. The hiring of Mr. Mark Garfield from the NINCDS, an individual with experience in handling proteins, but not in sequence analyses, as a replacement has kept the facility operational despite down time required for training on the protein sequencers. The Peptide Synthesis Facility has experienced similar personnel turnover problems and the recent loss of Mr. James Hoffman will surely have an impact on productivity until a replacement can be hired and trained. The capabilities of the Flow Cytometry Facility were enhanced by the arrival of Mr. Jeffrey Hooley to run the BD FACstar Plus. In the research area, Dr. Frits Koning left Dr. Coligan's laboratory this year to assume a tenured position at the Department of Immunohaematology and Bloodbank, University Hospital, Leiden, The Netherlands. Two new members have joined Dr. Coligan's laboratory during the past year: Dr. Kevin Moulder of the London School of Hygiene and Tropical Medicine and Dr. Gregory Einhorn of the Department of Molecular Biology and Genetics, Johns Hopkins University.

BIOLOGICAL RESOURCES BRANCH
Annual Report
October 1, 1987 to September 30, 1988

HONORS AND AWARDS

Dr. Coligan was invited to present laboratory data seminars at the Sloan Kettering Memorial Cancer Institute, University of Texas, University of Florida and University of Alabama. He was also invited to make presentations at the FASEB meeting in Las Vegas, the HLA-H2 Cloning Workshop that was held at Airlie House in Virginia and the meeting on the Molecular Basis of the Immune Response in New York City. Dr. Coligan was also recently invited to serve on the Program Committee for the American Association of Immunologists. Dr. Coligan was invited to serve on the editorial board of Molecular Immunology and continues to serve on the editorial board of Immunologic Research. Dr. Kevin Holmes made a presentation at the meeting on Mechanisms in B Cell Neoplasia held at the NIH.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00169-11 BRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of Murine and Human Transplantation Antigens and Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: John E. Coligan Branch Chief BRB/NIAID
OTHER: Diane Handy Staff Fellow BRB/NIAID

COOPERATING UNITS (if any)

W. Biddison, NINCDS, NIH; D. Margulies, NIAID, NIH; K. Ozato, NICHD, NIH

LAB/BRANCH

Biological Resources Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

2.6

PROFESSIONAL:

1.2

OTHER:

1.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

Major histocompatibility complex encoded class I molecules are integrally involved in the presentation of viral antigens to cytotoxic T lymphocytes (CTL). The goal of these studies is to gain an understanding of the regulation of expression, and the functional and antigenic properties of these molecules. It has been shown that multiple class I molecules can be generated from single genes by alternative splicing of pre-mRNAs. Particular alternatively spliced gene products affecting the C-terminus have been shown to distinguish H-2K molecules from H-2D molecules. For H-2K molecules, the splicing pattern favoring the use of a long exon 8 could be correlated with a potentialariat branch point adenosine 28 bp upstream of the splice junction. Elimination of this adenosine altered the pattern of pre-mRNA splicing in H-2K molecules resulting in a predominance of short exon 8 encoded sequences. The splicing pattern of H-2D region molecules could not be correlated with this base alone.

Previous studies have suggested that MHC class I molecules bind and present peptides to CTL, and that the peptides bind to two α helices in the class I molecule consisting of residues 50-84 and 138-180. For the M1 peptide of the influenza virus matrix protein, it was shown that the amino acid substitutions at positions 152 and 156 of HLA-A2.1 in the second α helix are important for recognition by influenza virus restricted CTL. However, it was shown that the failure to recognize complexes between the peptide and these mutant HLA-A2.1 molecules was due to T-cell receptor specificity and not the failure of the HLA-A2.1 molecules to bind peptide. For CTL recognition of HLA-A3 class I molecules the nature of the amino acid difference at position 152 was shown to be critical for distinguishing HLA-A3.1 and HLA3.2 molecules either by allo-immune CTL or virally restricted CTL. Different viruses and target cells did not alter this recognition pattern. Thus, both of these studies emphasize that the region between residues 150 and 160 of class I molecules as being critical for antigen presentation to cytotoxic T lymphocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00352-06 BRB																					
PERIOD COVERED October 1, 1987 to September 30, 1988																							
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Characterization of Cell Surface Molecules Important for Immune Function																							
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: John E. Coligan</td> <td style="width: 30%;">Branch Chief</td> <td style="width: 30%;">BRB/NIAID</td> </tr> <tr> <td>OTHER: Frits Koning</td> <td>Visiting Fellow</td> <td>BRB/NIAID</td> </tr> <tr> <td>Angel Ezquerro</td> <td>Visiting Fellow</td> <td>BRB/NIAID</td> </tr> <tr> <td>Kevin Moulder</td> <td>Visiting Fellow</td> <td>BRB/NIAID</td> </tr> <tr> <td>Gary Kikuchi</td> <td>NRC</td> <td>BRB/NIAID</td> </tr> <tr> <td>Gregory Einhorn</td> <td>IRTA</td> <td>BRB/NIAID</td> </tr> <tr> <td>Tom McConnell</td> <td>Guest Worker</td> <td>BRB/NIAID</td> </tr> </table>			PI: John E. Coligan	Branch Chief	BRB/NIAID	OTHER: Frits Koning	Visiting Fellow	BRB/NIAID	Angel Ezquerro	Visiting Fellow	BRB/NIAID	Kevin Moulder	Visiting Fellow	BRB/NIAID	Gary Kikuchi	NRC	BRB/NIAID	Gregory Einhorn	IRTA	BRB/NIAID	Tom McConnell	Guest Worker	BRB/NIAID
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Gregory Einhorn	IRTA	BRB/NIAID																					
Tom McConnell	Guest Worker	BRB/NIAID																					
COOPERATING UNITS (if any) E. Shevach, LI, NIAID; Georg Stingl, Vienna University, Austria; Jeff Bluestone, University of Chicago																							
LAB/BRANCH Biological Resources Branch																							
SECTION Office of the Chief																							
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892																							
TOTAL MAN-YEARS: 5.1	PROFESSIONAL: 3.8	OTHER: 1.3																					
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews																							
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Multiple molecules exist on the cell surface of lymphocytes that are important for development, activation and regulation of the immune response. Of prime importance in this regard is the clonotypic $\alpha\beta$ heterodimer T cell receptor (TCR) that is noncovalently associated with the multicomponent CD3 complex. In studies on the biosynthesis and assembly of TCR-CD3 complex, it has been shown that large amounts of non-disulfide linked α- and β-chains are synthesized most of which are degraded before assembly into a native TCR-CD3 complex. Final processing of the TCR-CD3 complex takes place only after complete assembly of the incompletely processed TCR and CD3 chains. A model for TCR-CD3 assembly and final processing was proposed. Analyses of the $\gamma\delta$ TCR revealed significant clonal diversity in expression indicating that the $\gamma\delta$ TCR has the potential to recognize a variety of ligands. Furthermore, the activation properties of cells bearing $\gamma\delta$ TCRs was found to be very similar to those bearing $\alpha\beta$ TCRs in terms of CD3 components and responsiveness to various activation signals such as anti-Thy-1, anti-CD3, PMA and ConA. The majority of $\gamma\delta$ expressing cells present in the periphery were found to be thymic dependent with a small percentage being thymic independent. Multiple forms of $\gamma\delta$ TCRs were found to be present in the periphery including those containing several types of γ4 chains. In addition, whereas $CD3^+$, $CD4^+$, $CD8^+$, $\alpha\beta$ TCR$^+$ cells have been found in the thymus, none could be detected in the periphery.																							

NOTICE OF INTRAMURAL RESEARCH PROJECT

201 AI 00543-01 BRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Recognition of Peptidic Antigens by Virus-Specific Cytotoxic T Lymphocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: W. Lee Maloy
OTHER: Robert W. Anderson
John E. Coligan

Senior Investigator BRB,NIAID
IRTA Fellow BRB,NIAID
Senior Investigator BRB,NIAID

COOPERATING UNITS (if any)

Bill Biddison, NI, NINCDS; D. Margulies, LI,NIAID

LAB/BRANCH

Biological Resources Branch

SECTION

Synthetic Peptide Antigen Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

2.7

PROFESSIONAL

1.5

OTHER:

1.2

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

It has been previously shown that MHC restricted cytotoxic T lymphocytes recognize foreign antigen in the form of short peptides. In defining the minimal requirements for the induction of virus specific CTL, a peptide containing epitopes from the influenza type A matrix (M1) protein recognized by both human CTL and T helper cells has been shown to sensitize target cells for recognition by influenza A virus specific CTL. This peptide was also shown to induce cytolytic effector cells from human peripheral blood lymphocytes (PBL) when cultured with the PBL even in the absence of exogenous growth factors. These data indicate that virus specific CTL can be stimulated in vitro with the CTL epitope and the lymphokines secreted by the T helper cells induced by the T helper cell epitope. This information will be important for the design of synthetic peptide based vaccines for the production of CTL mediated immunity.

To define the parameters of interaction of viral peptide antigens with MHC class I molecules, it was necessary to define peptide antigens recognized in the context of H-2 class I molecules available in soluble form i.e. H-2K^b and H-2D^d. The influenza A virus basic polymerase 2 (PB2) was found to be recognized by H-2D^d restricted influenza virus specific CTL, and of the thirty one peptides synthesized, three peptides from PB2 were found to be recognized by the virus specific CTL. These CTL epitopes have low amphipathicity values, but fit the Rothbard algorithm for T cell epitopes. The peptides that have been identified have been radiolabeled and the MHC molecule binding studies have begun. In addition, four epitopes recognized by SV40 specific, H-2D^b restricted CTL have been identified and studies on H-2K^b are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00172-10 BRB
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Synthesis of Peptide Antigens		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: W. Lee Maloy Senior Investigator BRB, NIAID		
COOPERATING UNITS (if any) John E. Coligan, BRB, NIAID; Louis Miller, LPD, NIAID; Ronald Schwartz, LI, NIAID; David Margulies, LI, NIAID, Mario Gorziglia, LID, NIAID		
LAB/BRANCH Biological Resources Branch		
SECTION Synthetic Peptide Antigen Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 0.7	PROFESSIONAL: 0.7	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) During the last year over 450 peptides have synthesized for 50 investigators in the NIAID. Many of the peptides have been made for collaborative projects. In collaboration with David Margulies, tritium labeled peptides for binding studies with MHC class I molecules have been synthesized. Antipeptide sera have been made for the continued study of the human and mouse T cell receptor and CD3 molecules in collaboration with John Coligan and his collaborators. In collaboration with Louis Miller, the immune response to the malaria parasite has been analyzed. Peptides have been used to help determine that the major epitope recognized by T helper cells in the circumsporozoite protein is variable between different variants of <i>Plasmodium falciparum</i>. In addition to the T helper recognized epitope, peptides have been used to define an epitope recognized by cytotoxic T cells in the circumsporozoite protein. In collaboration with Ron Schwartz the definition of a second epitope recognized by T helper cells in the cytochrome C molecule has been completed. Fifty-three variants of this epitope were synthesized in order to define the amino acid residues that interact with the T cell receptor and those that interact with the MHC class II molecule. In collaboration with Mario Gorziglia peptides and antipeptide sera were used to define epitopes in the VP7 and VP3 proteins of human rotavirus that are recognized by neutralizing antibodies.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00522-01 BRB
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Protein Sequence Analyses		
PRINCIPAL INVESTIGATOR (Last other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: John E. Coligan Branch Chief BRB/NIAID		
COOPERATING UNITS (If any) A. Lewis, LIP, NIAID; D. Margulies, LI, NIAID; W. Paul, LI, NIAID; L. Miller, LPD, NIAID; S. Venkatesan, LMM, NIAID; J. Battey, NCI; Ken Rock, Harvard Medical School		
LAB/BRANCH Biological Resources Branch		
SECTION Molecular Structure Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 1.3	PROFESSIONAL .2	OTHER 1.1
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) During the past year, the amino acid sequence of over 220 proteins and peptides has been determined by automated protein sequence analyses. In the case of several malaria proteins and a schistosome protein, the facility has purified tryptic peptides for sequence analysis by reverse phase microbore HPLC separation. Studies in which these analyses have had a major impact are listed below: (1) the characterization and identification of the putative <i>gag-pol</i> protease and <i>gag</i> encoded gene products of human immunodeficiency virus (HIV) (Laboratory of Molecular Microbiology); (2) the characterization of human pro-gastrin-releasing peptides expressed in baculovirus (Navy Medical Oncology Branch, NCI); (3) the identification of previously unidentified vaccinia virus encoded proteins (Laboratory of Viral Diseases); (4) the characterization of high-efficiency purified interleukin 4 (Laboratory of Immunology); (5) the structure of T-cell activating protein (TAP) and related Ly-6 encoded molecules (Harvard Medical School); (6) the identification of a chimeric MHC class II/class I protein expressed on the cell surface (Laboratory of Immunology); (7) protein sequence determinations which allowed the development of oligonucleotide probes for the cloning of PFs25 that is expressed on zygotes and ookinets of <i>P. falciparum</i> (Laboratory of Parasitic Diseases); (8) the identification of hamster MHC class I antigens (Laboratory of Immunopathology); (9) analysis of the BCDF (II-6) receptor (Laboratory of Immunoregulation); (10) identification of Dengue virus pre-matrix structural protein (Laboratory of Infectious Disease).		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-AI-00523-01-BRB
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Flow Cytometry Analysis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Kevin Lee Holmes Senior Staff Fellow BRB, NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Biological Resources Branch		
SECTION Flow Cytometry Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 5.2	PROFESSIONAL: 5	OTHER: 4.7
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☐ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The Flow Cytometry Facility has undergone considerable change in the last fiscal year. It has incorporated two new flow cytometers, hired and trained 3 new employees and instituted a new computer support contract for the section. All 12 IRP Laboratories have utilized the facility, using approximately 2158 hours of instrument time for single- and multi-parameter analysis and sorting. In addition, the user-operated instruments are generating an average of 30 files/day (approx. 1-2 hrs/day). The Flow Cytometry Facility has provided support to all 12 laboratories in the IRP and many non-NIAID labs, and therefore has impacted on a wide range of research projects. These include: 1) immunology: T and B cell ontogeny, effects of interleukins/biological response modifiers on lymphoid cells, B and T cell differentiation and physiology 2) virology: AIDS related research, murine leukemia viruses, murine immunodeficiency retrovirus, vaccinia virus; 3) parasitology: defense mechanisms to parasite invasion; 4) molecular biology: study of a variety of genes transfected into cultured cells. As the reliability of the instrumentation/personnel has become established in the facility, the frequency of repeat experiments has diminished while at the same time there has been an increase in the number of new users and projects. We expect that the level of use will increase further, particularly for sorting and multiparameter analysis. This increase was anticipated and influenced the decision to purchase the FACStar Plus and analyzers.		

Laboratory of Cellular and
Molecular Immunology
1987 Annual Report
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PHS-NIH
Summary Statement
Office of the Chief
Laboratory of Cellular and Molecular Immunology
October 1, 1987 through September 30, 1988

Introduction

The Laboratory of Cellular and Molecular Immunology was created in November, 1986. Its major task is to perform research on thymus-derived (T) lymphocytes. Its objective is to understand how these critical cells of the immune system differentiate and function. The approach is to define problems in whole animal systems, set up *in vitro* analogs in tissue culture, and determine the molecular basis for the phenomena. The laboratory employs techniques from the disciplines of cellular immunology, molecular biology and protein biochemistry.

During its second year the laboratory has pursued three major areas of research: 1) Antigen processing and presentation, 2) The biochemical basis of T cell activation and inactivation, and 3) The intrathymic events in T cell differentiation.

Antigen Processing and Presentation

T lymphocytes recognize foreign antigens in association with gene products of the major histocompatibility complex (MHC) on the surface of a second cell type known as an antigen-presenting cell (APC). The foreign antigen is usually not recognized in its native conformation. Rather, it is internalized by the APC and degraded into small peptides, which become associated with the MHC molecules inside the cell. The complexes of peptide and MHC molecule then move to the cell surface where they are recognized by antigen-specific receptors on T lymphocytes. At the present time the exact pathway followed by the antigen inside the cell is not clearly understood; nor are the cellular compartments in which the degradation and binding of the peptides takes place unequivocally delineated. Furthermore, the minimal requirements for peptide binding are not fully understood.

In this project we have first attempted to localize where inside the antigen-presenting cell antigen processing takes place. Variants of CHO cells, possessing temperature sensitive mutations in their ability to acidify endosomes, but not lysosomes, were transfected with MHC class II genes to allow them to present antigens to T cell hybridomas. At the nonpermissive temperature these cells were impaired in their ability to present intact foreign antigens, which require processing, but not in their ability to present peptide fragments, which do not. These results suggest that the endosome is one intracellular organelle where antigen processing takes place. In a related study with these cells, we demonstrated that uptake via the mannose-6-phosphate receptor of the precursor of cathepsin L, an acid protease, could interfere with antigen processing of molecules whose determinants were cleavable by the enzyme when the two were administered simultaneously. This observation argues that antigen passes through an acidic compartment during some phase of antigen processing. Finally, we have functionally mapped the structure of a second T cell antigenic determinant, identifying residues that interact with the MHC class II molecule, and residues that interact with T cell receptor. An analysis of the spatial relationship of these residues suggests that the determinant does not assume a pure alpha helical secondary structure when bound to the MHC class II molecule.

(K. McCoy, Z. Kovac, B. Beverly, and R.H. Schwartz; LCMI/NIAID, S. Gal and M. Gottesman; LMB/NCI; and W.L. Maloy; BRB/NIAID).

The Biochemical Basis of T Cell Activation and Inactivation

The ability of an organism to discriminate self from nonself molecules is a learned biological event. Clones of lymphocytes that are capable of reacting to self components are deleted or suppressed through an unknown *in vivo* mechanism. The goal of this project is to understand the cellular and biochemical mechanisms underlying this process using defined *in vitro* model systems. Stimulation of interleukin-2 (IL-2) producing T cell clones with antigen and MHC class II molecules (Ia molecules) in planar lipid membranes or on chemically-modified antigen-presenting cells (APC) results in a state of long lasting proliferative non-responsiveness to subsequent antigen stimulation in the presence of normal APC. Our recent experiments have shown that the induction of this state takes place when T cell receptor occupancy occurs in the absence of a critical costimulatory signal delivered by the APC. When this signal is provided in the form of allogeneic APC, it prevents the induction of nonresponsiveness and stimulates the T cell to proliferate. A biochemical analysis of second messenger signals inside the cell revealed that the costimulatory signal does not pass through protein kinase c activation nor through the calcium-dependent calmodulin-mediated pathway, both of which are stimulated by T cell receptor occupancy. These results suggest that the T cell requires stimulation via a third biochemical pathway in order to make a full proliferative response. Lack of the third signal results in the induction of the nonresponsive state.

(M. Jenkins, K. Hannestad, D. Mueller, R.H. Schwartz; LCMI/NIAID, J. Ashwell; BRMP/NCI)

T Cell Differentiation

This project is aimed at defining and characterizing the series of developmental events involved in the generation of mature, immunocompetent T cells, and the selective events which determine the antigen-recognition repertoire of these cells. These studies primarily utilize the thymus, the major site of T cell differentiation in the animal. In the past we have identified a number of thymocyte cell types and made possible the isolation of these cells at very high purity on a preparative scale. We have used these isolated cell types in an effort to define the state of maturation, precursor-product and lineage relationships, expression of T cell receptors, and the mechanisms and inductive signals responsible for growth, differentiation, and selection in the thymus.

During the past year we have further characterized the thymic TCR $\gamma\delta$ -bearing cells. Repopulation kinetic studies using grafted thymuses into nude mice revealed that there is a major thymic-dependent pathway as well as a minor thymic-independent pathway responsible for generating cells bearing this receptor. The studies in which hybridomas were made from thymic TCR $\gamma\delta$ thymocytes revealed new γ and δ species not detected in whole populations of CD4-8- thymocytes. Functional analysis of the hybridomas suggested that $\gamma\delta$ receptors used CD3-mediated activation pathways similar to those employed by TCR $\alpha\beta$ + T cells. A TCR $\alpha\beta$ + double negative thymocyte was also identified. These cells have many of the characteristics of mature T cells but are distinguished by a restricted TCRV β repertoire. Intrathymic transfer of isolated subsets of thymocytes into irradiated recipients revealed that this TCR $\alpha\beta$ + population can be derived from CD4+8- thymocytes. In strains of mice in which TCRV β 8.1-bearing thymocytes are deleted as a means of removing potentially self-reactive T cell clones, the TCR $\alpha\beta$ + double negative thymocytes from these mice actually show an enrichment for cells using the V β 8.1 receptor. These results suggest a mechanism for intrathymic tolerance generation whereby potentially autoreactive single positive thymocytes down-regulate their accessory molecules and become CD4-8- $\alpha\beta$ +. Finally, results obtained with *in vivo* anti-CD4 antibody treatment support the hypothesis that CD4+8+ thymocytes are the precursors of single positive thymocytes and that self tolerance by clonal deletion occurs at the CD4+8+ stage of development.

(B.J. Fowlkes, D. Pardoll, F. Ramsdell, and R.H. Schwartz; LCMI/NIAID)

Administrative, Organizational, and Other Changes

During the past year, Drs. Ephraim Fuchs, Steven Stringfellow, Paul Bacon, and Albert Bendelac, as well as Ms. Lynda Chiodetti and Ms. Susan Kirst joined the laboratory. Drs. Kathy McCoy, Marc Jenkins, Drew Pardoll, and Kristian Hannestad as well as Ms. Gina Stievenart left the laboratory.

Honors, Awards, and Scientific Recognition

Dr. Schwartz is a member of the editorial board of *Science*, *International Immunology*, *Immunology Today*, *The Journal of Molecular and Cellular Immunology*, and the *International Journal of Cell Cloning*. He was an invited speaker at the University of Pennsylvania annual Biomedical Graduate Studies Program Symposium, and at Juntendo University, Tokyo, Japan, for a symposium entitled, "Frontiers in Immunology". He was an invited speaker in a workshop on "The Molecular Basis of Antigen Presentation" held in Paris, France, a symposium at the 27th Midwinter Conference of Immunology in Asilomar, California, a participant in the symposium on "Molecular Mechanisms of Lymphoid Cell Interaction and Application to Tumor Immunology" held in Oahu, Hawaii, a speaker in the course "Seminars in Immunology" at UCSD, La Jolla, California, a speaker in the symposium on "The Immune System, 1988" at Schloss Elmau, West Germany, a visiting professor at Tuft's University Medical School, Boston, MA, a minisymposium chairman at the Federation of American Societies for Experimental Biology (FASEB) annual meeting in Las Vegas, Nevada, a seminar speaker at the University of Toronto in Canada, an invited symposium speaker at the Third European Network of Immunology Institutes Conference held in Les Embiez, France, chairman of a session in the workshop on the Immunology of Lysozyme held at NIH, and a speaker in the Gordon Conference on Immunochemistry and Immunobiology in Andover, New Hampshire.

Both Drs. Dan Mueller and Marc Jenkins were minisymposium speakers at the FASEB annual meeting in Las Vegas, Nevada. Dr. B.J. Fowlkes presented at the Immunochemistry and Immunobiology Gordon Conference in Andover, NH, and was an invited seminar speaker at the Department of Biology, Massachusetts Institute of Technology; the Department of Microbiology and Immunology, University of Rochester; the Department of Microbiology and Immunology, Syracuse University; the Department of Pathology, University of New Mexico; the Department of Pathology, Ohio State University; the Department of Pathology, University of Pennsylvania; the Departments of Microbiology and Biochemistry, George Washington University, and the Jackson Memorial Laboratories.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00529-01 LCMI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antigen Processing and Presentation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R.H. Schwartz	Chief	LCMI, NIAID
Others:	K. McCoy	Staff Fellow	LCMI, NIAID
	Z. Kovac	Visiting Associate	LCMI, NIAID
	B. Beverly	IRTA	LCMI, NIAID

COOPERATING UNITS (if any)

M. Gottesman and S. Gal, LMB, NCI
W.L. Maloy, BRB, NIAID

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5 1/2	3 1/2	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

We attempted to localize where inside the antigen-presenting cell antigen processing takes place. Variants of CHO cells, possessing temperature sensitive mutations in their ability to acidify endosomes, but not lysosomes, were transfected with MHC class II genes to allow them to present antigens to T cell hybridomas. At the nonpermissive temperature these cells were impaired in their ability to present intact foreign antigens, which require processing, but not in their ability to present peptide fragments, which do not. These results suggest that the endosome is one intracellular organelle where antigen processing takes place. In a related study with these cells, we demonstrated that uptake via the mannose-6-phosphate receptor of the precursor of cathepsin L, an acid protease, could interfere with antigen processing of molecules whose determinants were cleavable by the enzyme when the two were administered simultaneously. This observation argues that antigen passes through an acidic compartment during some phase of antigen processing. Finally, we have functionally mapped the structure of a second T cell antigenic determinant, identifying residues that interact with the MHC class II molecule, and residues that interact with the T cell receptor. An analysis of the spatial relationship of these residues suggests that the determinant does not assume a pure alpha helical secondary structure when bound to the MHC class II molecule.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00485-02 LCMI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

T Cell Activation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R.H. Schwartz	Chief	LCMI, NIAID
Other:	M.K. Jenkins	Staff Fellow	LCMI, NIAID
	K. Hannestad	Visiting Scientist	LCMI, NIAID
	D. Mueller	Medical Staff Fellow	LCMI, NIAID

COOPERATING UNITS (if any)

J. Ashwell, BRMP, NCI

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

4 1/2

PROFESSIONAL:

3 1/2

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Stimulation of interleukin-2 (IL-2) producing T cell clones with antigen and MHC class II molecules (Ia molecules) in planar lipid membranes or on chemically-modified antigen-presenting cells (APC) results in a state of long lasting proliferative nonresponsiveness to subsequent antigen stimulation in the presence of normal APC. Our recent experiments have shown that the induction of this state takes place when T cell receptor occupancy occurs in the absence of a critical costimulatory signal delivered by the APC. When this signal is provided in the form of allogeneic APC, it prevents the induction of nonresponsiveness and stimulates the T cell to proliferate. A biochemical analysis of second messenger signals inside the cell revealed that the costimulatory signal does not pass through protein kinase c activation nor through the calcium-dependent calmodulin-mediated pathway, both of which are stimulated by T cell receptor occupancy. These results suggest that the T cell requires stimulation via a third biochemical pathway in order to make a full proliferative response. Lack of the third signal results instead in the induction of the nonresponsive state.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00486-02 LCMI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT: 80 characters or less. Title must fit on one line between the borders.

T Cell Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)

PI:	BJ Fowlkes	Senior Investigator	LCMI, NIAID
Others:	Drew Pardoll	Medical Staff Fellow	LCMI, NIAID
	Fred Ramsdell	IRTA Fellow	LCMI, NIAID

COOPERATING UNITS (if any)

Ada Kruisbeek, Senior Investigator; BRMP, NCI

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

Section on Thymocyte Differentiation

INSTITUTE AND LOCATION

National Institutes of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

4

PROFESSIONAL:

3

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|---|
| ☐ (a) Human subjects | ☐ (b) Human tissues | ☒ (c) Neither |
| ☐ (a1) Minors | | |
| ☐ (a2) Interviews | | |

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

We have further characterized various cell types in the mouse thymus by biochemical, phenotypic, functional and/or differentiation potential. We have shown that T cell receptor (TCR) $\gamma\delta$ + CD4-8- (double negative) thymocytes are the major source of such cells in the peripheral lymphoid organs. A minor extrathymic pathway also exists for generating $\gamma\delta$ + T cells. The TCR- $\gamma\delta$ on thymocytes is associated with the same CD3 components and is activated by the same stimuli that activates TCR $\alpha\beta$ T cells. A TCR $\alpha\beta$ + double negative thymocyte was also identified. These cells have many of the characteristics of mature T cells but are distinguished by a restricted TCRV β repertoire. Intrathymic transfer of isolated subsets of thymocytes into irradiated recipients revealed that the TCR $\alpha\beta$ + cells can be derived from CD4+8- thymocytes. In strains of mice in which TCRV β 8.1-bearing thymocytes are deleted as a means of removing potentially self-reactive T cell clones, the TCR $\alpha\beta$ + double negative thymocytes show an enrichment for cells using the V β 8.1 receptor. Finally, results obtained with *in vivo* anti-CD4 antibody treatment support the hypothesis that CD4+8+ thymocytes are the precursors of single positive thymocytes and that self-tolerance by clonal deletion occurs at the CD4+8+ stage of development.

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Summary of Report
LABORATORY OF CLINICAL INVESTIGATION
October 1, 1987 to September 30, 1988
Michael M. Frank, M.D., Chief of Laboratory
and Clinical Director, NIAID

INTRODUCTION

This has been a fine year for the Laboratory of Clinical Investigation. Several new members of the staff have joined the laboratory in this period. Dr. Tom Leto has joined the Bacterial Diseases Section and Dr. Robert Hohman the Allergic Diseases Section, Dr. David Melnick, although not formally a member of the Laboratory of Clinical Investigation has come to participate in many of the patient care activities of the Bacterial Diseases group. There have been notable achievements in the research area. These will be discussed sequentially under each of the section topics.

Infectious Diseases Program

Medical Virology Section:

The Medical Virology program has had a number of notable discoveries during the course of the year. Possibly most important involves the nature of latency. Viruses which have the characteristic of latency are able to enter cells and persist for prolonged periods of time. They may be eventually expressed and cause disease at a much later period. Thus an individual who has varicella as a child may harbor latent virus and express this virus decades later at the time of lowered resistance in the form of herpes zoster infection. It has been known that in this situation the virus is localized in dorsal root ganglia and when re-expressed cutaneous disease tends to be expressed in a dermatome distribution, reflecting the distribution of nerves from the infected ganglion. In the past year, there has been analysis of both varicella and herpes simplex latency in human trigeminal ganglia. The technique used was in situ hybridization. A novel class of RNA transcribed from the herpes simplex genome in latently infected human ganglia has been noted. Dr. Straus and his group can now detect these latent transcripts by northern hybridization analysis as well. Basically, the group has been able to demonstrate that several of the early antigens coded for by the virus play an important role in the latency process. Although these antigens which this virus codes for are not expressed, the alternate DNA codons or anti-sense protein is expressed in the latent virus. Since it is known that such anti-sense protein may bind to the positive transcript, this suggests that latency in this case is triggered by a "turn off switch" mechanism. Of great importance is the finding that the virus is located in the neuronal cells of this ganglion. The nature of the transcripts is being defined. Varicella virus has been also found by in situ hybridization in these same trigeminal ganglia. Unlike herpes simplex these viruses have been found in accessory cells and not in the neurons. The nature of latency in this case appears to be different since much of the viral genome is transcribed rather than a very restricted number of genes.

The group has also made important strides in its studies of genital herpes in normal and immune impaired patients. They currently have data on five years of suppressive acyclovir therapy in patients with frequent recurring herpes genitalis. Studies have shown the drug to have remained effective, to be well tolerated and

not to induce viral resistance. After a long period of treatment some patients have less frequent recurrences. The role of herpes virus as a possible cofactor in the progression of pathogenesis of acquired immune deficiency syndrome (AIDS) is also being investigated. Using a model system, with part of the HIV virus genome inserted into indicator cells, it has been possible to show that herpes virus infection turns on HIV transcription. The biochemical mechanism for this effect is currently being resolved. Presumably, herpes simplex virus infection stimulates the release of an active gene product that is required for HIV long terminal repeat directed transcription.

Bacterial Diseases Section:

Drs. Malech, Gallin and the group of the Bacterial Diseases Section have also made noteworthy progress during the past year. A major focus of the group has been in studies of chronic granulomatous disease, since this represents an inherited cellular defect that has the possibility of being defined in molecular terms. Moreover, a large population of patients with chronic granulomatous disease is followed by the section, allowing studies to be done at the NIH that could not be done elsewhere. The group has shown that chronic granulomatous disease (CGD) is a heterogeneous group of diseases resulting from defects in H_2O_2 production by phagocytes. The sex linked form of the disease results from a defect in the gene coding for a 91kD subunit of cytochrome B, an enzyme required for oxidative function of phagocytes. In the past year, the group has shown that different kindreds have different abnormalities of this gene. Some patients make no RNA message coding for the protein; others make message but no protein and a third group make a functionally defective protein. Recently the group has also shown that there are two forms of cytochrome B positive autosomal recessive varieties of CGD. The most common form is associated with a missing 47kD cytosolic protein whose phosphorylation is important in activation of neutrophils. It is likely that the molecular pathology of this disease will be well understood shortly.

Further work continues on the differentiation of neutrophils with the development of monoclonal antibodies that recognize subpopulations of these cells. The use of such monoclonal antibodies will help decide whether changes in the types of cells circulating in various inflammatory conditions reflects differentiation of one group of cells or subgroups of cells capable of different neutrophil functions. Much of the work of the group has involved studies of receptor function on phagocytes. Phagocytes have receptors for interleukin-2 and it is interesting that the group found that patients with AIDS who are receiving interferon- γ or interleukin-2 differ in the number of secondary infections noted, although both cytokines are capable of stimulating neutrophils. Patients receiving interferon- γ had markedly fewer bacterial infections than those receiving interleukin-2. The effect of interferon- γ on neutrophils and monocytes from patients with CGD was also studied. It is fascinating to note that interferon- γ corrected the bactericidal defects in the phagocytes of many CGD patients. This occurred in some individuals who did not develop an oxidative burst because they had no cytochrome B, suggesting that mechanisms for bacterial killing, other than the oxidative burst, can be activated by interferon- γ . This raises important therapeutic considerations in the treatment of this unfortunate group of patients. It was also shown that interferon- γ downregulates hyperimmunoglobulin-E synthesis in cells of patients with the hyperglobulin-E frequent infection (Job's syndrome). Interferon- γ was also shown to cause granuloma formation in vitro. Of course, granulomas represent one of the major toxic manifestations of chronic granulomatous disease.

One of the major transduction mechanisms in the neutrophil membrane is represented by G proteins. These proteins interact with receptors and GTP to transduce signals and upregulate or downregulate cell function. A chemotactic factor receptor as well as one of the inhibitory G proteins are present and functionally coupled in an intracellular pool in neutrophils. An antibody has been developed that recognizes cytochrome B. This antibody shows that most of the protein is intracellular in resting neutrophils and translocates to the surface with activation. Two proteins, one of 47kD and one of 65kD are essential for transduction effects in neutrophils leading to superoxide production. Interestingly, the cytoskeletal protein fodrin was found to be the major calmodulin binding protein in neutrophils.

Clinical Mycology Section:

The Clinical Mycology group has continued its studies of fungal pathogenesis. Human alveolar macrophages obtained by bronchial lavage were found to bind *Aspergillus fumigatus* conidia by lectin-like interactions that are inhibited by monosaccharides and glycans. At least two different receptors were involved in this binding. An attempt is being made to raise antibodies against such receptor proteins. The group has been very interested in amphotericin B packaged in liposomes. In various models these have been found to be far less toxic than the amphotericin B that we give to patients with fungal infection but equally effective. Blood and tissue levels of these packaged antibiotics are similar to the levels found with the usual dosage regimen. Presumably, the liposomal preparations will concentrate the drug in macrophages and other phagocytic cells where it may be far more effective. Further studies of this material are under way.

Dr. June Kwon-Chung has continued her studies of the molecular biology and antigenic variation of both cryptococci of various types and candida species. The mitochondrial genome size of two varieties of *Cryptococcus neoformans* were found to vary between 40 and 45 kilobases. Previously the group had shown two phenotypic varieties of candida and had gone on to show that these two varieties differ in their pathogenicity. Now a genetic basis for this difference has been discovered which may allow more sophisticated analysis of pathogenesis to take place.

Immunology Program

Clinical Immunology Section:

The immunology program of the Laboratory of Clinical Investigation in many ways dovetails with the microbial pathogenesis program. Dr. Louis Fries, in the past year, has studied the role of complement in parainfluenza type 3 infection. In the parainfluenza virus studies it has been shown that certain IgM monoclonal antibodies to the hemagglutinin-neuraminidase protein can produce neutralization in the presence of an active complement cascade. This activity is totally dependent on viral lysis and must proceed the classical complement pathway. Interestingly, the virus itself is a spontaneous activator of the classical pathway and binds the same components of complement to its surface. However, this binding does not lead to viral lysis in the absence of specific antibody directing complement assembly. The model is important in understanding the early steps of viral neutralization and also important in understanding the molecular basis of how antibody causes complement component assembly on a particle surface. Studies of herpes simplex

virus type I have shown that the viral genome causes synthesis of a protein which acts as a C3 fragment receptor. Dr. Fries and his group have shown that isolated glycoprotein responsible for this activity is a potent modulator of complement mediated cytolysis in vitro and has demonstrated several mechanisms for this effect. In more recent work they have shown that herpes simplex type II also induces expression of a C3b binding protein like HSV1 but this protein has totally different regulatory properties with respect to the complement cascade. Working with Dr. Harvey Friedman at the University of Pennsylvania a stably transfected murine cell line which expresses herpes simplex virus protein gC under control of an inducible promoter has been developed. These systems are being used to study the binding site for C3b on the protein and the biological consequences of a C3 binding protein in the cell membrane. Presumably such a protein will prevent the cell from attack by immunologic mechanisms thereby protecting the virus.

In a second major area, the interactions of C3b with immunoglobulin G have been further studied. Earlier studies showed that IgG antibody is an excellent acceptor for activated C3b and tends to form a stable complex. It has been shown that such stable complexes are far more effective in killing bacteria than are either antibody or complement alone. The reason for this is in part because C3b bound to IgG is in a protected site and cannot be inactivated by control proteins as it is normally and also because the antibody directs complement attack. In the past year high affinity interactions involving C3b-IgG heterodimer and phagocytic cells have been studied. It has been shown that individual molecules of IgG become far more effective in pathophysiologic terms when they are bound to single molecules of C3b. C3b-IgG complexes display unique opsonic activity not reproduced by C3b and IgG randomly located on the surface of a particle. Studies of the behavior of the C3 receptor have also been conducted during this year. These studies have shown that the receptor cycles through a pre-lysosomal compartment after the cell is activated. Surprisingly it has been shown that multi-valent preligation of CRI of human macrophages significantly upregulates subsequent IgG-mediated phagocytosis of particles coated with IgG. The mechanism for this is currently under study.

An exciting development of the section this year has been the discovery of a new plasma protein present in relatively large concentrations in human serum. This 120kD glycoprotein is a poor antigen explaining its failure to be identified thus far. This protein specifically binds to inactivated C4 and has many similarities to C2. At this point it appears to have domains in common with C2 but it is present even in C2 deficient human serum. The protein acts as a regulator of the classical complement pathway and can inhibit both C1 and C2 interactions in the classical pathway as well as C3 site formation. The biochemistry and functional significance of this new plasma protein are currently under study.

Previous studies from the group had shown that scavengers of the oxidative burst can upregulate phagocytic Fc receptor function on granulocytes. Methionine and taurine, which block n-chloramines and hypochlorous acid respectively were shown to increase the ingestion of IgG coated cells by three to five fold. These inhibitors also increased the adherence of IgG antibody coated targets to neutrophils while having no effect on adherence of C3 coated targets. These results further suggest that the long-lived, toxic products of myeloperoxidase (MPO) halide system are primarily responsible for downregulation of neutrophil phagocytosis. These products appear to interfere with the function of PMN FcR but

not PMN CR1. Studies this year have also detailed the phagocytic function of neutrophils of patients with acute bacterial infections. They have shown that such Fc receptor function is markedly upregulated and longitudinal studies have shown that this upregulation coincides with the duration of infection. Studies of Fc receptor number have shown that FcR1 increase dramatically during acute infection but that Fc II and III do not. Interestingly, neither CR1 nor CR3 complement receptors increase in number on the surface of cells during infection. However, CR3 but not CR1 are capable of phagocytosis by these infected cells. Studies have also been performed of patients with angioedema, particularly an acquired form. These patients have been shown to synthesize antibody to one of their own plasma proteins, the C1 esterase inhibitor. Work this year has shown that such antibodies not only influence the complement cascade but prevent normal regulation of the kinin generating system as well. Interestingly, their effects on kallikrein are much greater than their effects on Hageman factor suggesting that different sites on C1 inhibitor may be involved in each of these inactivations or that the antibodies do not recognize the precise site of inactivation. Moreover, they suggest that the clinical illness in these patients is more likely related to kallikrein dysregulation than Hageman factor dysregulation.

Mucosal Immunity Section:

The mucosal immunity section has also had important scientific advances during the past year. An enormous amount of work has been done in defining at the cellular and molecular level the signalling events in IgA heavy chain class switching. In the normal GI tract cells committed to making IgM antibody can be switched to making IgA. These studies have used a specific antigen reactant B cell line as well as normal purified immunoglobulin M(+) B cells. Studies with recombinant lymphokines disclosed that IL-4 induces an increase in the percentage of sIgA (+) cells to 6-15% with minimal effects on IgA secretion. On the other hand, IL-5 causes increased IgA secretion but has no effect on expression. Addition of both lymphokines causes increased IgA expression as well as increased IgA secretion, suggesting that it is the sequential effect of both lymphokines that causes the switching. Other lymphokines do not have this effect. Complementary studies with normal B cells obtained from Peyer's patches; patches of lymphocytes within the bowel, show that sIgA (+) B cells are induced by IL-5 to secrete increasing amounts of IgA. It will be of importance to find out whether IgA-B cells can be caused to switch their secretory profile by IL-4 treatment. The group has also had great success in studying the profile of cells formed during various kinds of inflammatory gastrointestinal disease. For example, in studies of primary biliary cirrhosis (PBC) a particular population of cells that are CD4(+) Leu8(+) T cells from patients with this disease do not suppress immunoglobulin synthesis as do cells from normal individuals. In normal individuals treatment of such cells with anti-Leu8 antibody enhances their suppressor function. This does not occur in patients with primary biliary cirrhosis. Interestingly, the proliferative response of CD4(+) Leu8(-) T cells from patients with PBC and controls were similar but the proliferative response of CD4(+) Leu8(+) T cells from patients with PBC were lower than those of controls. This suggests that a defect in the cell population may play a critical role in the development of this disease.

Studies of non-human primate bowel lymphocytes have also continued. It has been shown that circulating T cells respond to IL-4 while lamina propria T cells do not. The latter cells however do respond to rIL-2 and to the mitogen conA. Other studies have shown the ability of such cells supernatants to stimulate

proliferation of various types of B cells. These studies show for the first time that two major lymphoid compartments of the intestinal immune system co-exist in the mesenteric lymph nodes and that these compartments differ in their response to lymphokines.

Studies of the expression of Leu-8 in various cells during the differentiation process and when activated have also been underway. Many of these stimuli cause a decline in Leu-8 expression but other stimuli do not lead to such loss of expression. This loss of Leu-8 expression is reversible. Interestingly, with the loss of Leu-8 positivity T cells continue to express their suppressor function suggesting that this molecule, though an important recognition molecule for identifying these cells is not the critical molecule in maintaining their suppressor activity. The microenvironment of the GI tract is obviously of critical importance in determining expression and function of these cells.

Allergic Diseases Program

Allergic Diseases Section:

Dr. Michael Kaliner's group has been involved in many projects designed to explore the pathophysiologic mechanisms responsible for the development of allergic disease. Studies have ranged from examination of biochemical events in lung tissue to studies of the skin. For example, it was found that in guinea pig lung, epithelial cells and smooth muscle cells were the most responsive cells to isoproterenol as evidenced by changes in cyclic AMP. This drug obviously is of great importance in the treatment of asthma. Patients with gram negative shock were studied for the appearance of histamine in their blood. Plasma histamine levels in these patients was found to be normal or below normal. An inhibitor of the enzyme responsible for the formation of histamine was found in these patients. Skin histamine was found to be high in patients developing vibrational angioedema, showing that it is possible to measure histamine levels in human beings during cutaneous attacks of disease. New assays were developed for histamine and other biologic mediators of immediate hypersensitivity. In studying these mediators it was shown that the gaps that appear between endothelial cells that appear to allow leakage of fluid from the intravascular space to the extravascular space are larger than 140 Å but are the same for all of the various mediators studied.

The neuropeptides have been under investigation in this laboratory for a number of years. It was suggested that neurogenic stimulation of sensory nerves leads to increased cutaneous microvascular permeability with these agents but does not involve mast cell degranulation.

Human neutrophils release a factor which causes rat RBL cells to release histamine. This histamine releasing factor from neutrophils has been further characterized. It has been shown to activate cutaneous human mast cells in vivo and in vitro. The factor has been shown to have one fraction with the molecular size of approximately 2500. It acts independently of IgE and can be differentiated from other early histamine releasing factors. It seems likely that this factor may participate in human late phase allergic reactions.

Anaphylaxis associated with menses in women has been a particular interest to this group over the years. Subgroups of patients have been defined and four individuals responsive to an LH-RH analog have been treated. The pathophysiology

of this disease is under further study at present. Dr. Hohman has been studying rat basophil leukemia cells (RBL cells) and their degranulation events as a way of understanding the molecular events which occur during this process. Methods have been developed for permeabilizing cells in such a way that water soluble agents can be introduced into the cell causing degranulation without killing the cells and without separating proteins that must be in contact to allow the degranulation events to take place. It will be possible to study such cells to determine intermolecular relationships required for the degranulation process.

Studies of nasal mucosa physiology have also been in progress. Rhinitis is the common final pathway of many allergy events. Provocation of nasal mucosa causing protein secretion has been studied in detail to determine which proteins are secreted actively and which are simply a product of endothelial cell leakage of plasma proteins. Histamine was found to cause increased vascular permeability and this could be blocked with an antihistamine. Methacholine on the other hand caused selective granular secretion as reflected by increases in total protein, secretory IgA, lysozyme, lactoferrin and IgG. These effects were inhibited by atropine.

Mast Cell Physiology Section:

Dr. Metcalfe's group continues to study mast cell growth and differentiation. Human bone marrow, under the influence of IL-3 and in suspension was shown to give rise to 10 to 30% basophils and these could be cultured for 2 to 3 weeks under the appropriate conditions. Granulocyte-macrophage colony stimulating factor was shown to inhibit this IL-3 effect. The proteoglycans of human mast cells were further characterized. Interestingly, it was shown that products of the oxidative burst degrade these proteoglycans.

Dr. Metcalfe and his group now have the largest population of patients with mastocytosis to be followed anywhere. They have been able to develop a new classification of this disease and new methods of treatment. They are currently involved in a double blind study comparing ketotifen, a mast cell stabilizing drug, and antihistamines in the treatment of one of the milder forms of this disease. The entire clinical spectrum of the disease has been defined and the types of therapy suitable for each stage of the illness have been further identified. Staging techniques including bone marrow studies, bone scans and radiographic studies have been utilized.

Studies of the diagnosis and treatment of adverse reactions to foods and additives have also continued. It has been shown that patients with shrimp allergy have allergy to three substances, one of which is a tRNA from the shrimp. This antigen plus two glycoproteins are responsible for shrimp hypersensitivity leading the way to production of improved diagnostic and therapeutic methods of treatment. Studies of aspartame hypersensitivity have not revealed problems with this important food additive.

Honors and Awards:

Two members of the Laboratory of Clinical Investigation, Drs. Harry Malech and Dean Metcalfe, were elected to the American Society for Clinical Investigation this year. At this point, all tenured members of the Laboratory of Clinical Investigation are members of the ASCI, the highest honorary society for those involved in clinical investigation.

Dr. Stephen Straus received the meritorious service award from the Public Health Service and Dr. Ken Croen, of that section, has received the American Society of Microbiology Young Investigator Award.

Dr. Warren Strober received the Public Health Service Distinguished Service Award. Dr. Warren Strober was elected Chairman of the Board in Allergy/Immunology, replacing Dr. Michael Kaliner the previous Board Chairman.

Dr. John E. Bennett was appointed to the Subspecialty Board in Infectious Disease of the American Board of Internal Medicine.

Dr. Michael Frank delivered the 16th Max Finland Lecture in Infectious Disease at Harvard Medical School and was appointed Chairman of the Scientific Advisory Board of the Leonard Wood Memorial, American Leprosy Foundation. Dr. Frank is block chairman for the area of complement for the Federation of Societies in Experimental Biology and Dr. Kaliner is block chairman for the Immediate Hypersensitivity Group.

Dr. John Gallin received the highly prestigious Squibb Award of the Infectious Disease Society of America as well as a meritorious service award from the Public Health Service. He received a Doctor of Science, Honoris Causa, Amherst College, May 29, 1988 and was elected vice-president of the International Society of the Immunocompromised Host.

Dr. Harry Malech was elected a member of the European Society for Clinical Investigation. Dr. Michael Frank was asked to serve on the directorate for the International Congress of Immunology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00043-23 LCI
PERIOD COVERED Oct. 1, 1987 to Sept. 30, 1988.		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Immunology and Chemotherapy of Systemic Mycoses</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: John E. Bennett, Head, Clinical Mycology Section LCI, NIAID Others: Edward McManus Medical Staff Fellow LCI, NIAID Virginia Kan Guest Worker LCI, NIAID Francoise Dromer Visiting Fellow LCI, NIAID		
COOPERATING UNITS (if any) 1. Pulmonary Disease Division (Dr. Yaeger) and Lombardi Cancer Center (Dr. Rhaman) Georgetown University Hospital, Washington, D.C. 2. Dept. of Medicine, Tufts University School of Medicine, Boston, MA (Dr. Gelfand)		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Clinical Mycology Section		
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Md.		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) Human alveolar macrophages obtained by bronchial lavage were found to bind <i>Aspergillus fumigatus</i> conidia by lectin-like interactions that were inhibitable by monosaccharides and glycans that represent at least two different receptors. These did not include the complement receptors but did include lectins recognizing beta glucan and chitin-like saccharides. Polyclonal antibody is being raised against a similar family of receptors on the murine macrophage cell line, J774A1, in order to facilitate purification of receptor proteins. Liposomal amphotericin B of very small particle size was found to be less toxic but equally effective as desoxycholate amphotericin B in a murine model of cryptococcosis. Blood and tissue concentrations of the liposomal amphotericin B were also measured in animals and found to be comparable to the desoxycholate complexed drug. Using another preparation of liposomal amphotericin B, release of IL-1 and TNF from human blood mononuclear cells was less with liposomal than desoxycholate amphotericin B, offering an explanation for reduced pyrogenicity observed with the liposomal product. In experimental disseminated murine sporotrichosis, two new triazole compounds were found to be effective but an allylamine, terbinafine, was not effective. In an study of the immunogenicity of different preparations of rabbit IgG, differences in glycosylation could not be detected and did not account for the differing immunogenicity in mice.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00045-20 LCI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Interaction of Antibody and Complement on Production of Immune Damage

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Michael M. Frank, Chief, LCI/NIAID

Others: Dr. Louis Fries, Senior Staff, NIAID

Gilda Linton, LCI/NIAID

Karen Fenstamaker, LCI/NIAID

Dr. Carl Hammer, Senior Staff, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

2.7

PROFESSIONAL

2.2

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A recently defined subgroup of patients with acquired angioedema (AAE) were found to elaborate autoantibodies to C1 inhibitor (C1-Inh). The C1-Inh molecule is important in regulating C1 activation, but also represents the major control mechanism influencing the enzymes of the contact system: activated Hageman factor (XIIa), Hageman factor fragment (XIIa), and plasma kallikrein (KK). Since uncontrolled action of these enzymes could promote both C1-Inh consumption and generation of the vasoactive peptide bradykinin, we have examined the impact of the AAE C1-Inh autoantibodies on the control of the contact enzymes KK and XIIa by normal plasma C1-Inh.

Endogenous plasma kallikrein showed enhanced survival in AAE plasmas similar to that seen in untreated hereditary angioedema (HAE) plasmas. Mixing HAE or AAE plasmas with normal plasma revealed partial correction of the HAE plasma defect but little or no change of the AAE plasma defect. This finding implies active inhibition of C1-Inh function by AAE plasmas.

When immunoglobulin concentrates from AAE plasmas were mixed with normal plasmas the kinetics of exogenous KK inhibition demonstrated progressive, dose responsive retardation and the concentrates induced KK inhibitory defects like those observed in HAE plasmas.

In marked contrast defects in XIIa inhibition obtained at immunoglobulin doses simulating patient plasmas were small relative to those found in HAE plasma and in general there was less than a two-fold prolongation of XIIa survival. Enzyme-linked differential antibody immunoabsorbent assays confirmed that all three patient antibodies had more profound effects on KK-C1-Inh complex formation than on complex formation between C1-Inh and factor XIIa-derived enzymes.

These studies indicate that the autoantibodies elaborated by AAE patients can cause dysregulation of the contact system as well as the complement cascade. This derangement in control of the contact system could contribute to the consumption of C1-Inh and production of angioedema in these patients.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00047-19 LCI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Studies of Patients with Known or Suspected Parasitic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.A. Ottesen	Head, Clinical Parasitology Section	LCI/LPD, NIAID
	T.E. Nash	Senior Investigator	LCI/LPD, NIAID
	F.A. Neva	Chief	LPD, NIAID
		Senior Investigator	LCI, NIAID

Others: See next page

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Parasitology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of these studies is to increase understanding of the pathogenesis of parasitic diseases and to improve their diagnostic, therapeutic and preventative measures. Investigations have been carried out in patients both at NIH and in the endemic areas of Guatemala (onchocerciasis), India (filariasis, kala azar), Mexico and Honduras (leishmaniasis), and Sudan (schistosomiasis).

Diagnostic refinements have been made with immunoassays for filarial infections (based on IgG₄ antibodies) and for strongyloidiasis (based on immediate hypersensitive skin tests) and with clinical measures (ultrasonography) for schistosomal liver fibrosis. Studies on pathogenesis have shown that there is antigenic variation in giardia parasites during the course of experimental infections in humans; also IgG₄ antibodies appear to be the "blocking antibodies" controlling allergic responsiveness to parasite antigens in infected individuals. Therapeutic studies with ivermectin have shown it to be at least as effective and safe at low dosages in bancroftian filariasis as the currently used drug diethylcarbamazine (DEC), even after a single oral dose, and to be the only drug demonstrated effective in mansonellosis. A double-blinded, placebo controlled trial of weekly DEC in Peace Corps volunteers going to West Africa has demonstrated that acquisition of loiasis by travellers can be prevented by such chemoprophylaxis.

Other Professional Personnel

T. Nutman	Senior Investigator	LPD, NIAID
R. Hussain	Visiting Fellow	LPD, NIAID
A. Cheever	Assistant Chief	LPD, NIAID
K. Colina	Howard Hughes Fellow	LPD, NIAID
R.G. Crystal	Chief	PB, NHLBI
D. Sacks	Staff Fellow	LPD, NIAID
P. Melby	NRSA Fellow	LPD, NIAID
D. Freedman	Medical Staff Fellow	LCI, NIAID
E. Pearce	Visiting Fellow	LPD, NIAID
J. Ruthlein	Guest Researcher	LCI, NIAID
R. Adam	Medical Staff Fellow	LPD, NIAID
R. Davey	Medical Staff Fellow	LCI, NIAID
A. Aggarwal	Visiting Fellow	LPD, NIAID
S. James	Senior Investigator	LCI, NIAID
W. Rom	Visiting Associate	PB, NHLBI
C. Chan	Senior Investigator	CB, NEI
R. Nussenblatt	Chief	CB, NEI
P. Brindley	Visiting Fellow	LPD, NIAID
M. Ballard	Medical Staff Fellow	LPD, NIAID

Cooperating Units

University of Arizona (E. Petersen); Peace Corps Medical Office, Washington, DC (M. Mulligan); Department of Health, Guatemala (G. Zea-Flores); University of Michigan (J. Bennett); University of Khartoum, Sudan (M. Homeida); Harvard University, Boston, MA (S. Ackerman, F. Von Lichtenberg); Onchocerciasis Chemotherapeutic Research Center, Tamale, Ghana (K. Awadzi); Indian Council of Medical Research Tuberculosis Research Center, Madras, India (S.P. Tripathy, R. Prabhakar, V. Kumaraswami, and V. Vijayan); Madras Medical College, Madras, India (K. Vijayasekaran); Institute of Dermatology, Santa Domingo, Dominican Republic (H. Bogaert); Instituto de Salubridad y Enfermadades Tropicales, Mexico City, Mexico (O. Velasco); Rajendra Memorial Institute, Patna, India (A.B. Sen); Walter Reed Army Institute of Research, Washington, DC (J. Chulay, W. Ballou, W. Hockmeyer, S. Hoffman, J. Berman); SEMA, Inc., Rockville, MD (G. Phillips); Merck, Sharpe Dohme, Rahway, NJ (D. Neu); University of Maryland, Baltimore, MD (M. Levine; D.A. Harrington, G.A. Losonsky, S. Savarino); Pvt. Consult (B. Walton); Ministry of Health, Tegucigalpa, Honduras (C. Ponce); Dept. of Biology, Youngstown University, Ohio (R. Kreutzer); and Yale University School of Public Health, Dept. Epidemiology, New Haven, CT (D. McMahon-Pratt).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZOI AI 00048-18 LCI
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Pathophysiology of Autoimmune Hemolytic Anemia		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Michael M. Frank, Chief, LCI/NIAID Others: C. Hammer, Senior Investigator, LCI/NIAID L. Fries, Medical Officer (Research), LCI/NIAID M. Marques, Visiting Fellow, LCI/NIAID P. Langlois, IRTA M. Basta, Visiting Fellow, LCI/NIAID		
COOPERATING UNITS (if any) 		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Clinical Immunology Section		
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 3	PROFESSIONAL: 3	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) In the past year attention has returned to the studies of antibody and complement in the clearance of erythrocytes from the circulation. Specific questions include the reasons for the difference between effects of IgG and IgM antibodies. IgG antibody causes splenic sequestration at low doses, IgM antibody causes hepatic sequestration. In both cases, complement is required for this effect. A second question is the complement receptors involved in such clearance mechanisms. Major findings this year include the unexpected finding that on a per complement fixing site basis, IgM antibody deposits far more C3 on a target than does IgG. The kinetics of deposition are also different; C3 deposition with IgM is far faster. Once bound to the target, C3 is shed from both the IgG and the IgM coated targets at the same rate. The molecular form of C3 deposited on the target has been found to be C3bi with both IgM and IgG with very little C3b on the target. In a guinea pig model there is little evidence for the formation of C3dg in vitro on the erythrocyte surface. The fact that some C3b is deposited on the target can be shown by experiments in which sensitized cells are treated with trypsin. Trypsin is known to preferentially degrade C3bi. Once trypsinized, erythrocytes sensitized with antibody can be shown to have small amounts of C3b on their surface obscured by large amounts of C3bi. In preliminary experiments, such trypsinized cells which have released most of their C3 from the surface are cleared from the circulation in guinea pigs to the same extent as cells not treated with trypsin. These preliminary data suggest that C3b is the major opsonin causing clearance. Firming up these various points and determining the absolute number of ligands in each clinical situation is our major goal at present.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00057-15 LCI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Basic Studies on Pathogenic Fungi

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: K. J. Kwon-Chung

Research LCI, NIAID
Microbiologist

Other: W. L. Whelan, A. Varma

COOPERATING UNITS (if any)

NIDR

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

3.5

PROFESSIONAL

2.0

OTHER

1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Topics of current studies include 1) cloning of mitochondrial DNA sequences of Cryptococcus neoformans; 2) isolation of antigenic variants of Candida albicans; 3) determination of ploidy and karyotype of Candida stellatoidea; 4) comparison of Cladosporium trichoides, an etiologic agent of mycotic brain abscess, with Xylohypha nigrescens.

In current studies, we have determined that the mitochondrial genome size of the two varieties of Cryptococcus neoformans range between 40-45 kilobases. Some cloned mt DNA fragments ranging between 1 to 1.8 kb showed variety specific patterns of homology when they were hybridized with total DNA digests of numerous isolates obtained from various parts of the world. These findings supported previous results that the phenotypic differences between the two varieties are based on their genetic differences. A method, without mutagenesis, was developed to isolate antigenic variants from serotype A as well as B isolates of Candida albicans.

arl4lci.rpt

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00058-14 LCI
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pathogenesis and Chemotherapy of Herpesvirus Infections in Man		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	S. E. Straus	Senior Investigator LCI, NIAID
	J. Ostrove	Senior Staff Fellow LCI, NIAID
	K. Croen	Medical Staff Fellow LCI, NIAID
	M. Sawyer	Medical Staff Fellow LCI, NIAID
	A. Freifeld	Medical Staff Fellow LCI, NIAID
	R. Holman	Medical Staff Fellow LCI, NIAID
	P. Krause	Medical Staff Fellow LCI, NIAID
	J. Dale	Clinical Research Nurse LCI, NIAID
COOPERATING UNITS ~~of~~ <u>San</u> Nagpal Visiting Fellow LCI, NIAID		
J. Rooney, Senior Staff Fellow, LOM, NIDR; A. Notkins, Chief, LOM, NIDR; S. Nussinoff-Lehrman (Burroughs Wellcome Company), Y. Bryson (UCLA)		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Medical Virology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 4.5	PROFESSIONAL: 3.5	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The goals of the project are to characterize the pathogenesis, the natural history and the therapy of herpes simplex virus and varicella zoster virus infections. Our clinical emphasis has been on genital herpes in normal and immune-impaired patients. Analysis of five years of suppressive acyclovir in patients with frequently recurring herpes have shown the drug to remain effective, to be well tolerated, and to not induce drug resistance. We have shown for the first time that with prolonged observation some individuals diminish their rates of herpetic recurrences and no longer warrant chronic suppressive therapy. We continue to utilize our human model of recurrent herpes simplex virus infection. We are conducting detailed studies of ultraviolet light and other physical and chemical inducers of recurrent skin infection. Open trials of agents designed to block the induced reactivation have been initiated. A placebo-controlled trial of acyclovir for blocking ultra-violet induced reactivation is nearly complete. We have also initiated a placebo-controlled trial of acyclovir for suppression of frequent spontaneously recurring oral herpes. The major thrust of our laboratory effort in this project has been the analysis of herpes simplex latency in human trigeminal ganglia using in situ hybridization we recently demonstrated a novel class of RNAs transcribed from the HSV genome in latently infected human ganglia. We can now detect these latency transcripts by northern hybridization analysis as well. Recent detailed studies have identified three latency transcripts whose termini have been more accurately mapped. Because of the great importance of these findings for an understanding of human HSV infection, we began a series of projects aimed at identifying the mechanism of action of the latency associated gene.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00154-13 LCI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

Events in Immediate Hypersensitivity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID
Others:	Martha V. White, M.D.	NRSA Fellow	LCI/NIAID
	Mary E. Revenis, M.D.	Guest Researcher	LCI/NIAID
	Marek L. Kowalski, M.D.	Fogarty Visiting Fellow	LCI/NIAID
	Alain Didier, M.D.	Special Volunteer	LCI/NIAID
	Diane Bradley	Chemist	LCI/NIAID

COOPERATING UNITS (if any)

Marc M. Friedman, Ph.D., Julie Goth, Ph.D., Georgetown University
(Contract # N0-1-A1-22665)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

1.2

PROFESSIONAL

0.4

OTHER

0.8

CHECK APPROPRIATE BOXES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided)

This broad-based project is designed to explore pathophysiologic mechanisms responsible for allergic diseases. During the past year, the following observations have been made: The identification of cells responding to isoproterenol with increases in cyclic AMP in guinea pig lung has been described. The presence of an inhibitor of histamine N-methyltransferase in plasma obtained from patients in septic shock has been described and the plasma levels of histamine in sepsis determined; septic plasma has normal histamine. The sequence of events in allergy skin testing has been determined, including definition of the morphology of cutaneous mast cell degranulation and concomitant changes in plasma histamine. The major clinical response to mast cell mediators is increased vascular permeability. The characteristics of vascular permeability in response to mediators, neurogenic inflammation, and late phase allergic responses have been explored. A molecule released from neutrophils which is capable of causing mast cell degranulation has been discovered and named HRA-N. HRA-N is 1400-2400 daltons, heat stable, active in vivo and in vitro, and may play a role in the generation of late phase allergic reactions. Tracheal aspirates of children with bronchopulmonary dysplasia have been found to be relatively deficient in lactoferrin, while having the same levels of histamine, albumin, and total protein as controls with hyaline membrane disease. This observation has potential importance in early identification of BPD-prone infants. Finally, the disease cataneneal anaphylaxis has been described and a treatment developed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00155-13 LCI
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Phagocytic Cell Function		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: John I. Gallin, M.D. Scientific Director, IRP, NIAID Others: Harry L. Malech, M.D. Head, Bacterial Dis. Sect. LCI/NIAID Karen Lomax, M.D. Senior Staff Fellow LCI/NIAID Daniel Rotrosen, M.D. Senior Staff Fellow LCI/NIAID Philip Murphy, M.D. Medical Staff Fellow LCI/NIAID Hiroyuki Nunoi, M.D. Visiting Associate LCI/NIAID Stuart Abramson, M.D., Ph.D. Med. Staff Fellow LCI/NIAID		
COOP. UNITS: J Nath, Dept. Hematol., Walter Reed Army Inst. Res., COOPERATING UNITS (if any) Washington, DC; B Seligmann, Ciba Geigy, Summit, NJ; S Dreskin, A&R, NIADDDK; CJ White, IRP/NIAID; W Zimmerli, Kantons- spital, Basel, Switzerland; Y Ohno, Tenri Hosp., Japan; R Lerher, Dept. Medicine, Univ. California Los Angeles, Los Angeles, CA.		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Bacterial Diseases Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 3.9	PROFESSIONAL: 3.1	OTHER: 0.8
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☒ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The major objective of this project has been to study <u>neutrophil function</u> in health and disease. Determination of the biochemical and <u>genetic</u> basis of inherited diseases affecting phagocytes and associated with <u>recurrent infections</u>, and the diagnosis and treatment of these diseases is a major component of this project. Also within the scope of this project is the study of in vitro model systems of these inherited diseases using preparations of phagocytic cells derived from normal volunteers and patients. We have shown that <u>Chronic Granulomatous Diseases</u> (CGD) are a heterogeneous group of diseases resulting from absent H2O2 production by phagocytes, associated with recurrent infections. The X-linked form results from a defect in the gene coding for the 91 kDa subunit of <u>cytochrome b</u>. We show that different kindreds have different lesions of this gene, in that some patients make no mRNA coding for this protein, others make mRNA but no protein, while some make a functionally defective protein. These observations suggest that the gene defect may be a new mutation in many kindreds and that mutations occur at different sites. We have recently shown that there are two forms of cytochrome b-positive, autosomal recessive CGD. The most common form is missing a 47 kDa cytosol protein, whose <u>phosphorylation</u> plays a role in <u>activation</u> of neutrophils <u>oxidative metabolism</u>. We have demonstrated that the lesion responsible for neutrophil <u>specific granule deficiency</u> is a differentiation stage specific lesion in myeloid cell <u>gene regulation</u>. In particular, there is a failure of <u>lactoferrin</u> mRNA production in myeloid cells, but lactoferrin is synthesized normally in nasal secretions of specific granule deficient patients.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00189-08 LCI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical and Biochemical Studies of Human Enteral Adenovirus Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S.E. Straus

Senior Investigator, LCI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

Laboratory of Clinical Investigation

INSTITUTE AND LOCATION

Medical Virology Section

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

NIAID, NIH, Bethesda, Maryland 20892

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Project Terminated

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00192-09 LCI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Immediate Hypersensitivity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Dean D. Metcalfe, M.D.	Head, Mast Cell Physiology Section	LCI/NIAID
Others:	Arnold S. Kirshenbaum, M.D.	NRSA	LCI/NIAID
	Beth Friedman, M.D.	Medical Staff Fellow	LCI/NIAID
	Helen Thompson, Ph.D.	Visiting Fellow	LCI/NIAID

COOPERATING UNITS (if any)

The University of Washington (C.W. Henderson and S. Klebanoff)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mast Cell Physiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Terminated

NOTICE OF INTRAMURAL RESEARCH PROJECT

301 AI 00249-07 LCI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Pathogenesis, Diagnosis, and Treatment of Systemic Mast Cell Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)

PI: Dean D. Metcalfe, M.D. Head, Mast Cell Physiology Section LCI/NIAID

Others: Brett V. Kettelhut, M.D. Medical Staff Fellow LCI/NIAID

Beth Friedman, M.D. Medical Staff Fellow LCI/NIAID

COOPERATING UNITS AND
LABORATORY, Department, NIH Clinical Center (Dr. William Travis); Hematology
Service, NIH Clinical Center (Dr. Robert Parker)LABORATORY
Laboratory of Clinical InvestigationSECTION
Mast Cell Physiology SectionINSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.00	1.75	0.25

CHECK APPROPRIATE BOXES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

A review of over 60 cases of adult mastocytosis followed at the NIH, has substantiated the observation that mastocytosis can occur in distinctly different forms, or categories. We have, thus, instituted a new classification for mast cell disorders. Diseases of mast cell proliferation are now divided as follows: indolent mastocytosis, mastocytosis with dysmyelopoiesis, mast cell leukemia, and lymphadenopathic mastocytosis with eosinophilia. Each type of disease varies from other categories in its prognosis and treatment.

Pediatric patients with urticaria pigmentosa or diffuse cutaneous mastocytosis often have systemic disease, as evidenced by the presence of a unique bone marrow lesion. These heretofore unrecognized lesions consist of focal collections of mast cells, eosinophils, and lymphocytes along small vessel endothelial cells.

A double blind crossover trial comparing ketotifen, a mast cell stabilizing drug, and an H1 antihistamine; and hydroxyzine, an H1 antihistamine, has shown hydroxyzine to be more effective at lowering symptom scores. Neither drug affected plasma or urine histamine levels.

Topical steroids lower the number of mast cells in skin and result in dramatic improvement in mastocytosis skin lesions.

Dermal mast cells are increased 15-20 fold in patients with mastocytosis, and 2-3 fold in patients with idiopathic anaphylaxis or flushing. Mast cells first increase around endothelial cells.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00250-06 LCI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical and Basic Studies on Inflammatory Diseases of the Gastrointestinal Tract

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Dean D. Metcalfe, M.D. Head, Mast Cell Physiology Section LCI/NIAID

COOPERATING UNITS (if any)

Indian Institute of Science, Bangalore, India (P.V. Subba Rao)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mast Cell Physiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.50

PROFESSIONAL:

0.25

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Terminated

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00269-07
LCI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neutrophil Subpopulations

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: John I. Gallin, M.D. Scientific Director, IRP, NIAID
Others: Harry L. Malech, M.D. Head, Bacterial Dis. Sect. LCI/NIAID
C. C. Brown, M.D. (Former) Medical Staff Fellow LCI/NIAID

COOPERATING UNITS (if any) P. Krause, Dept. Pediatrics, Hartford Hosp., Univ. Connecticut, Hartford, CT.; A. Segal, Hematology, Univ. Hospital, Univ. London, London, England.

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Bacterial Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

0.3

PROFESSIONAL

0.3

OTHER

CHECK APPROPRIATE BOXES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

(PROJECT TERMINATED) Human neutrophil heterogeneity has been suggested because of differences in functional capacity, surface receptors and antigenic markers. Heterogeneity of binding of monoclonal antibodies to human neutrophils was examined using the fluorescent activated cell sorter. We developed an IgG monoclonal antibody, 31D8, that binds strongly to neutrophils that are more responsive functionally, representing about 95% of normal neutrophils. Normal volunteers given 4 ng/kg E. coli endotoxin have an initial drop in total circulating neutrophils, but an absolute increase in the number of circulating neutrophils which bind 31D8 weakly (dull). This is followed by a dramatic increase in total neutrophils, but particularly in the number of 31D8 "dull" neutrophils. There is some correlation between circulating band forms and 31D8 "dull" antibody binding indicating a correlation with immaturity. 31D8 "dull" neutrophils increase after severe blunt trauma or after experimental infusion of endotoxin. Band forms and 31D8 weakly reactive cells comprise an overlapping but not identical population. 31D8 expression may be a measure of neutrophil maturation independent of nuclear morphology and may be useful to measure the degree of marrow release of neutrophils. Dr. Segal developed IgG monoclonal antibody, C10, which we have characterized as binding strongly to about 30 to 80% of neutrophils. Percent C10 positive neutrophils in the same individual is constant over months. Lack of C10 negative cells is seen in a patient with leukemoid reaction and lack of C10 positive neutrophils is seen in specific granule deficiency. C10 expression may play a role in control of myeloid proliferation and differentiation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00271-07 LCI
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Purification and Characterization of Complement Proteins and Fragments		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Carl H. Hammer, Senior Investigator, LCI/NIAID Others: Michael M. Frank, Chief, LCI/NIAID Ruth M. Jacobs, Medical Staff Fellow, LCI/NIAID Yannick Pilatte, Visiting Fellow, LCI/NIAID Paul Langlois, IRTA Milan Basta, Visiting Fellow, LCI/NIAID Louis F. Fries, Medical Officer, LCI/NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Clinical Immunology Section		
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.6	PROFESSIONAL: 3	OTHER: 1
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) We have isolated and partially characterized a previously unrecognized protease-sensitive plasma protein identified during the development of a new protocol for the purification of human C2. This new protein is physicochemically similar to C2. It co-precipitated with C2 on polyethylene glycol (PEG) fractionation and specifically and independently bound, like C2, to Sepharose bound iC4/C4b. Like C2 this protein was cleaved in plasma or serum to immunochemically distinct fragments of beta (90kDa) and alpha (30kDa) mobility that may lose the capacity to bind to iC4 and/or C4b. The purified protein has a chain structure similar to C2 as determined by SDS-PAGE in the presence or absence of reducing agent and has a molecular mass of 120 kDa only somewhat greater than C2 at 95 kDa. C2 has been shown to contain about 16% N-linked carbohydrates. Using independent techniques, we have identified 16.8% N-linked sugar in C2 and 14.2% in gp120 kDa although the glycosylation pattern was different in the two proteins. Both proteins were shown to contain 6% sialic acid. Both proteins radioiodinated under similar conditions to the same specific activities with each of two different methods that produced 10-fold disparate results. These proteins have immunochemical similarities as well. Although monospecific anti-serum to each did not cross-immunoprecipitate by standard gel techniques, anti-gp120 kDa serum did specifically identify shared epitopes by Western blot analysis. Mancini analysis identified 250-300 ug/ml of gp120 kDa in plasma. Initial studies suggested that the contact system protease Kallikrein is responsible for the rapid cleavage of gp120 kDa when stored in glass tubes at 4°C in normal and HAE plasmas. It will be important to confirm the protease responsible for gp120 kDa cleavage and to isolate the fragments to pursue biological and functional studies <u>in vitro</u> and in animals (see project #Z01 AI 00278-06 LCI). Since C2 is a member of a recently described class of C4b/C3b binding proteins which contain a 60-amino-acid repeat sequence, it will be of interest to determine if gp120 kDa is also a member of this class of proteins.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

201 AI 00272-06 LCI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (50 characters or less. Title must fit on one line between the borders.)

Host Defense Against Pneumococcal Bacteremia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Michael M. Frank, Chief, LCI/NIAID

Others: David Bobak, Medical Staff Fellow, LCI/NIAID

Ronald Washburn, Medical Staff Fellow, LCI/NIAID

Andrea Tenner, Scientist One, Dept. of Biochem., Amer. Red Cross

Biomedical Research and Development, Rockville, Maryland

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

1.1

PROFESSIONAL

1.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project has been inactive during the current year but we do not wish to discontinue it.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00275-07 LCI
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Complement Receptor and C3 Mediated Opsonization		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Thelma Gaither, Research Biologist Others: Michael M. Frank, M.D., Clinical Director Hank Simms, M.D., Research Fellow Thomas Quinn, M.D., Sr. Investigator Leslie Gofney, Biologist Divya Verma, Laboratory Worker		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Clinical Immunology Section		
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 2	PROFESSIONAL: 2.5	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) We have found that scavengers of products that are formed during the normal oxidative burst of polymorphonuclear leukocytes (PMN) can upregulate PMN phagocytosis. Methionine and taurine (which block n-chloramines and hypochlorous acid, respectively) increased the ingestion of IgG-coated sheep RBC (EiG) by 3 to 5 fold. Like azide and catalase (which block earlier steps in the oxidative burst) these inhibitors also increased the adherence of EiG to PMN, while having no effect on adherence of C3b-coated target cells. These results suggest that the long-lived, toxic products of myeloperoxidase (MPO)-H₂O₂-halide system are primarily responsible for downregulating PMN phagocytosis. These products appear to interfere with the function of PMN FcR, but not PMN CR1. We have shown that the phagocytic function of PMN of patients with acute bacterial infections is markedly upregulated, even in the absence of inhibitors of the oxidative burst. Longitudinal studies showed that the enhanced phagocytosis paralleled a reduction in superoxide anion production which was seen on the first day after hospital admission. Phagocytic activity and superoxide levels normalized as patients improved clinically. Enhanced phagocytosis in patient PMN was not associated with increased surface expression of the low-affinity PMN Fc receptors, FcRII or FcRIII; however, in 5 of 7 patients tested, there was a marked increase in the surface expression of high-affinity FcR (FcRI), which is not normally expressed on the surface of PMN. Unlike PMN, the extent of ingestion by patient monocytes (MO) was normal; however, kinetic studies showed that patient MO ingest EiG significantly more rapidly than normal MO. Thus the IgG-dependent phagocytic capacity of both MO and PMN of patients with acute bacterial infections is upregulated. Soluble inflammatory mediators may play an important role in the enhanced phagocytosis in patient PMN. We have found that IL-1, IL-2 and TNF each upregulate PMN phagocytosis in the absence of inhibitors of the oxidative burst. This effect was not related to increased expression of high or low-affinity FcR		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00278-07 LCI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the Components of the Complement Cascade

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Carl H. Hammer, Senior Investigator, LCI/NIAID

Others: Michael M. Frank, Chief, LCI/NIAID

Ruth M. Jacobs, Medical Staff Fellow, LCI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Disease, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

1.0

PROFESSIONAL

1.5

OTHER

1

CHECK APPROPRIATE BOXES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

We have modified our published procedure for the rapid isolation of the second component of human complement to obtain increased yield and specific functional activity. Also isolated by this new method is a protein which has many physicochemical properties in common with C2. See project #Z01 AI 00271-06 LCI for a detailed description of the isolation and partial characterization of this new plasma protein termed gp120 kDa. The capacity of this protein to specifically bind to iC4/C4b, the numerous similarities to C2 and its relatively high plasma concentration (250-300 ug/ml) strongly suggested that this sialoglycoprotein may participate in the classical complement pathway. Thus, initial attempts to delineate a functional role for gp120 kDa were directed to the early events of complement activation in this pathway. We used complement coated sheep erythrocytes with limited functional sites EAC4b or EAC1,4b as well as one or more other limited complement components to study the effect of gp120 kDa at different points in the formation of the classical pathway C5 convertase. By several distinct functional tests we were able to show dramatic inhibition (>90%) by gp120 kDa of classical pathway convertase formation. Inhibition was shown for C1, C2, and C3 site formation and up to 30% acceleration of C2 decay was observed. The exact point(s) where gp120 kDa exerts its influence has to be determined possibly by competition and binding experiments using radiolabeled proteins. These effects were obtained at input concentrations between 15 and 255 ug/ml of gp120 kDa. To assess the ability of gp120 kDa to functionally influence later steps in the complement sequence we also studied its affect on reactive lysis. Again this glycoprotein significantly inhibited complement mediated lysis both during and following EAC5b,6,7 site formation. Since we have shown the direct binding of gp120 kDa to surface bound iC4/C4b, we did expect an influence on the convertase formation. However, the ability of gp120 kDa to inhibit reactive lysis was unexpected and further work is needed elucidate the mode of action on the terminal complement components.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00279-07 LCI
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies on Mucous Glycoproteins		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Michael A. Kaliner, M.D. Head, Allergic Diseases Section LCI/NIAID		
COOPERATING UNITS (if any) James H. Shelhamer, M.D., Carolea Logun, and Jens D. Lundgren, Critical Care Medicine, Clinical Center		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Allergic Diseases Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Mucus secretion is a normal function of respiratory mucous membranes. Models for measurement of mucus production by cultured human and feline bronchial and nasal mucosae have been developed in order to examine the controls of mucus secretion. Corticosteroids inhibit MGP release by lowering baseline secretion. Analysis of corticosteroid treated airways reveals a close correlation between lipocortin generation and MGP production. Pulmonary inflammation with neutrophils is often associated with mucus production. Lysates of human neutrophils as well as supernatants from activated neutrophils cause airways to release MGP; this activity is not due to elastase, and identification of the mucus secretagogue is under study. Endorphins cause increased MGP secretion. Substance P is found in close association with submucous glands. Substance P causes MGP secretion, and the activity of substance P is markedly increased by protection from degradation by proteases with thiorphan. Platelet activating factor (PAF) activates feline airways to secrete MGP, and this action requires the formation of arachinoids and is inhibitable by CCS.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00354 06 LCI

PERIOD COVERED October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Immunoregulatory Defects in Inflammatory Bowel Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Stephen P. James, Investigator, Mucosal Immunity Section, LCI, NIAID

Others:

Warren Strober, Head, Mucosal Immunity Section, LCI, NIAID
Marjorie Kanof, NRSA Fellow, Mucosal Immunity Section, LCI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH Laboratory of Clinical Investigation

SECTION Mucosal Immunity Section

INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects	☒ (b) Human tissues	☐ (c) Neither
☐ (a1) Minors		
☐ (a2) Interviews		

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Previously we have shown that the intestinal lamina propria contains a unique population of activated CD4+ T cells which do not express the cell membrane glycoprotein Leu-8 and have high helper function. During FY 88 we have studied the relationship between cell activation and the expression of the Leu-8 antigen on human T cells. When resting peripheral blood lymphocytes were activated with PHA, anti-CD3 + PMA, or ionomycin + PMA, there was a rapid decline in expression of the Leu-8 antigen on all lymphocyte subpopulations. The change in Leu-8 expression was found to be dependent on the mechanism of cell activation, since an intermediate decline in Leu-8 expression occurred following stimulation with Con A and there was little loss of expression of Leu-8 after stimulation of lymphocytes with pokeweed mitogen or allogeneic lymphocytes. The decline in Leu-8 expression on activated lymphocytes occurred much earlier than the decline in expression of CD45R, another cell membrane glycoprotein which is modulated during cell activation. The change in Leu-8 expression was reversible, since peripheral blood T cells or CD4+ Jurkat cells rapidly re-expressed Leu-8 following removal of the activation stimuli. To study the relationship between Leu-8 expression and cell function, the expression of Leu-8 on peripheral blood CD4+, Leu-8+ T cells was reduced by prior activation with PHA, and it was found that these cells continued to exhibit suppressor function for PWM-stimulated immunoglobulin synthesis. Thus, there is a rapid decline in expression of the Leu-8 antigen but no change in regulatory function of CD4+, Leu-8+ T cells during cell activation. These results suggest that the molecule recognized by anti-Leu-8 plays a role in lymphocyte activation, but not directly in the effector function of CD4+, Leu-8+ T cells. These studies also suggest that the microenvironment of the intestinal lamina propria probably contains a factor or factors which are necessary for CD4 T cells to permanently differentiate into cells having helper phenotype and function. Further studies are in progress to define cytokines which may act as activation and differentiation factors which are necessary for critical host defense functions of CD4 T cells in mucosal tissues.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00355 06 LCI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunoregulatory Defects in Primary Biliary Cirrhosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Stephen P. James, Investigator, Mucosal Immunity Section, LCI, NIAID

COOPERATING UNITS (if any)

E. Anthony Jones, M.D., Chief, Section on Liver Diseases, NIDDK

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

(b) Human tissues

☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previously we have shown that patients with primary biliary cirrhosis (PBC) have abnormal regulatory function of T cells. To study further whether abnormalities of lymphocyte function in PBC are due to altered function of T cell subpopulations, phenotypic and functional characteristics of CD4+ T cells were examined. The proportion of CD4+ T cells expressing the Leu-8 and CD45R antigens was normal in patients with PBC. The capacity of CD4+, Leu-8- T cells to provide helper function for PWM-stimulated immunoglobulin synthesis by B cells *in vitro* was similar in patients and controls. However, in contrast to normal individuals and patients with other liver diseases, CD4+, Leu-8+ T cells from patients with PBC did not suppress immunoglobulin synthesis. Whereas treatment of CD4+ T cells from normal individuals with anti-Leu-8 monoclonal antibody enhanced their suppressor function, similar treatment of CD4+ T cells from patients with PBC did not increase their suppressor function. To determine whether the abnormal regulatory function of CD4+, Leu-8+ T cells was due to a defect of cell activation, the response of CD4+ T cell subpopulations to mitogenic stimulation was examined. The proliferative responses of CD4+, Leu-8- T cells from patients with PBC and controls were similar, but the proliferative responses of CD4+, Leu-8+ T cells from patients with PBC were lower than those of control cells. Since the CD4+, Leu-8+ T cell population plays a role in suppressing immunoglobulin synthesis and is contained within the autoreactive T cell population, the abnormal function of this T cell subpopulation in patients with PBC may play a central role in defective immunoregulation found in this disease.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00356-06 LCI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the Regulation of IgA Immunoglobulin Synthesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I. Warren Strober, M.D., Head, Mucosal Immunity Section, LCI/NIAID
Other Michael C. Sneller, M.D., Investigator, National Foundation for Ileitis
and Colitis, LCI/NIAID
Dennis Y. Kunitomo, M.D., Fellow Alberta Heritage Foundation, LCI/NIAID
Gregory R. Harriman, M.D., Medical Staff Fellow, LCI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

3.0

PROFESSIONAL

2.5

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The overall aim of this project is to define at both the cellular and molecular level, the signalling events involved in IgA heavy chain (C_H) class switching. Studies during this period focused on the regulation of isotype switching in the antigen (SRBC)-reactive B cell line CH12.LX as well as on normal, purified IgM(+) B cells. CH12.LX cells comprise a stable population of B cells bearing sIgM (98-99%) as well as a small population of cells bearing sIgA (1-2%). Studies with recombinant lymphokines disclosed that rIL-4 induces an increase in the percentage of sIgA(+) cells (to 6-15%) with minimal effects on IgA secretion, whereas IL-5 induced increased IgA secretion, but had no effect on sIgA expression. The addition of both lymphokines induced both increased IgA expression and IgA secretion. No effect on mIgA expression or IgA secretion was seen with other lymphokines including IL-1, IL-2, IL-3, IL-6, GM-CSF and IFN-γ. In further studies, it was shown that IL-5 causes IgA secretion by sIgA(+) but not sIgA(-) CH12.LX cells indicating that IL-5 is acting as a post-isotype switch differentiation factor. Thus, these studies indicate that IL-4 and IL-5 act in a sequential fashion to induce IgA expression and secretion in the CH12.LX system: IL-4 inducing differentiation of sIgM(+) cells to sIgA(+) cells and IgA enhances the IgA secretion of the resulting sIgA(+) cells.

Complementary studies with normal B cells (derived from Peyer's patches) utilized cells separated into sIgA(+) and sIgA(-) cells obtained by panning or sorting with by FACS. Here, LPS-driven sIgA(+) B cells were induced by rIL-5 to secrete increased amounts of IgA; in contrast, mIgA(-) cells manifested no such secretion. Thus, it could be shown that IL-5 acts on normal B cells as a post-switch factor, promoting differentiation of IgA B cell into IgA secreting plasma cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00357-05 LCI
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of the Autologous Mixed Lymphocyte Reaction		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P.I. Warren Strober, M.D., Head, Mucosal Immunity Section, LCI/NIAID Other Stephen P. James, M.D., Senior Investigator, Mucosal Immunity Section, LCI/NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Mucosal Immunity Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☒ (b) Human tissues ☐ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) 		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00397-05 LCI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Interactions of C3b with Immunoglobulin G-Regulation of C3b Function by Antibody

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Louis F. Fries, Medical Officer (Research), LCI/NIAID

Others: Michael M. Frank, Chief, LCI/NIAID

John Zurlo, Medical Staff Fellow, LCI/NIAID

Milan Basta, Visiting Fellow, LCI/NIAID

Marisa Marques, Visiting Fellow, LCI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

1.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

We have previously shown that complement fragment C3b interacts with IgG to form a stable covalent complex. We have demonstrated that formation of these complexes can proceed under plasma conditions and occurs on serum-treated soluble and particulate immune complexes, including bacterial pathogens. C3b residues in such complexes are protected from usual control mechanisms, support exaggerated alternative pathway activity, and are capable of high affinity interactions with human phagocytes which elicit internalization responses. C3b-IgG complexes display unique opsonic activity not reproduced by C3b and IgG randomly located on the surface of target particles.

During the last year, we have characterized the behavior the C3b receptor of polymorphonuclear leukocytes in states known to cause internalization. We have shown that this receptor recycles through a pre-lysosomal compartment unless multi-valently ligated. In an attempt to dissect the unique opsonic effects of C3b-IgG complexes, and C3b in general, we have shown that multi-valent preligation of CR1 in human macrophages significantly upregulates subsequent IgG-mediated phagocytosis of particles not bearing C3b. The role of specific IgG in "focusing" and amplifying complement fragment deposition, with resultant enhanced opsonization, phagocytosis, and killing of a human pathogen has been examined with *Cryptococcus neoformans*. Here, the presence of a small number of IgG residues on the cryptococcal capsule elicits a marked increase in phagocytosis and killing, but only if present at the moment of complement activation.

In an effort to probe the influence of non-immune ambient IgG on complement-mediated opsonization, we have also studied the observed complement opsonization defects in multiple myeloma sera and in impact of exogenous supraphysiologic IgG infusions on complement-dependent blood stream clearance in an in-vivo model.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00428-03 LCI
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) IgE Immunotoxins		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Michael A. Kaliner, M.D. Head, Allergic Diseases Section LCI/NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Allergic Diseases Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☐ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project has been terminated in 1988.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00429-04 LCI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Nasal Responses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID
Others:	Gordon D. Raphael, M.D.	Medical Staff Fellow	LCI/NIAID
	Aleksander Z. Gawin, M.D.	Special Volunteer	LCI/NIAID
	James N. Baraniuk, M.D.	Special Volunteer	LCI/NIAID
	Scott D. Meredith, M.D.	Special Volunteer	LCI/NIAID
	Diane Bradley	Chemist	LCI/NIAID

COOPERATING UNITS (if any)

Robert F. Bonner, M.D., Biomedical Engineering and Instrumentation Branch, Division of Research Services; and James H. Shelhamer, M.D., Critical Care Medicine, Clinical Center

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

1.7

PROFESSIONAL

1.5

OTHER

0.2

CHECK APPROPRIATE BOXES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The nasal mucosa is the first internal surface to encounter aeroallergens, airborne pathogens, and airborne toxins. Analysis of nasal responses will likely provide insights into normal host defense mechanisms. Provocation of human and guinea pig nasal mucosa stimulates protein secretion. Analysis of the components of these secretions has revealed new insights into the source of these secretions as well as possible mechanisms for the pharmacologic control of secretion.

Histamine was found to primarily cause increased vascular permeability as reflected in relative enrichment of nasal secretions with albumin, nonsecretory IgA (NS-IgA), and IgG. These effects were inhibited with an H-1 antihistamine but not by atropine. Methacholine caused selective glandular secretion as reflected in increases in total protein, secretory IgA (sIgA), lysozyme, lactoferrin, albumin, and IgG, but no relative enrichment of any of these proteins. Methacholine effects were inhibited by atropine.

Hot, spicy foods may cause rhinorrhea in sensitive subjects. Gustatory rhinitis was found to involve an atropine-inhibitable muscarinic pathway and both symptoms and secretory responses could be prevented by pretreatment with atropine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00430-04 LCI
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Biology of Varicella-Zoster Virus Infections		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	S.E. Straus J. Ostrove P. Krause K. Croen M. Sawyer A. Freifeld S. Nagpal	Senior Investigator Senior Staff Fellow Medical Staff Fellow Medical Staff Fellow Medical Staff Fellow Medical Staff Fellow Visiting Fellow LCI, NIAID LCI, NIAID LCI, NIAID LCI, NIAID LCI, NIAID LCI, NIAID LCI, NIAID
COOPERATING UNITS (if any)		
J. Hay (USUHS), W. Ruyechan (USUHS)		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Medical Virology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 4.0	PROFESSIONAL: 3.25	OTHER: 0.75
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
This project constitutes one of the two major laboratory research focuses of this section. The goals are to identify, map and characterize varicella-zoster virus genes and proteins, expressed during active, latent, or productive infections, and to define the temporal sequence and control of gene expression. To accomplish these ends we use an extensive array of molecular biological techniques. To date we have mapped the viral genome and most of its transcripts. We have identified and carefully mapped a number of important viral gene products. We defined the molecular basis for drug resistance to acyclovir in laboratory derived and human VZV strains. Recently, we proved that VZV remains latent in satellite cells of human sensory nerve ganglia and that some, but not all, VZV genes are expressed during latency. Our current effort is most heavily directed at better defining the nature and control of VZV gene expression in latently infected human tissues.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00432 04 LCI

PERIOD COVERED October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Regulation of Mucosal Immune Responses in Nonhuman primates

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Stephen P. James, Investigator, Mucosal Immunity Section, LCI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH Laboratory of Clinical Investigation

SECTION Mucosal Immunity Section

INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.9	PROFESSIONAL: 0.4	OTHER: 0.5
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CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

The proliferation, differentiation and regulatory function of T cells in the intestinal lamina propria is thought to depend largely on their production of cytokines. Previously we have shown in nonhuman primates that intestinal lamina propria (LPL) T cells have high expression of IL-2 receptors, have high capacity for IL-2 production, and produce helper factors for immunoglobulin synthesis in response to intestinal pathogens. In the present study we investigated the response of nonhuman primate T cells to human recombinant IL-4 and have examined their capacity to produce B cell proliferation factors. T cells from MLN exhibited a dose-dependent proliferative response (3H-thymidine incorporation) when cultured with rIL-4. The response was dependent on the presence of phorbol myristate acetate (PMA) as a co-stimulus. In contrast, LPL T cells had a minimal response to rIL-4, but did have high responses to rIL-2 and the mitogen concanavalin A. To investigate lymphokine production by T cells, culture supernatants from concanavalin A stimulated T cells were obtained. Using a Staph A Cowans (SAC) co-stimulation assay, it was shown that supernatants from both MLN and LPL T cells stimulate proliferation of MLN B cells. In addition, it was shown that mitogen activated LPL T cells produce substantially higher IFN- γ bioactivity and have much higher levels of IFN- γ mRNA than activated MLN T cells. These studies show for the first time that the two major lymphoid compartments of intestinal immune system, organized lymphoid cells in the mesenteric lymph nodes and diffusely scattered lymphocytes in the intestinal lamina propria, are fundamentally different in their production and utilization of the lymphokines IL-2, IL-4 and IFN- γ .

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00447-03 LCI
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Clq: Its Biosynthesis and Biological Functions		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Michael M. Frank, Chief, LCI/NIAID Others: David Bobak, Medical Staff Fellow, LCI/NIAID		
COOPERATING UNITS (if any) Andrea Tenner, Scientist I, Dept. of Biochemistry, American Red Cross Biomedical Research and Development, Rockville, MD		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Clinical Immunology Section		
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.2	PROFESSIONAL: 1.2	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project has been discontinued.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 201 AI 00469-03 LCI
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Complement: Studies in Viral Infection		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: Louis F. Fries, Medical Officer (Research), LCI/NIAID Others: Srikantan Vasantha, Visiting Fellow, LCI/NIAID Brian Murphy, Senior Investigator, LID/NIAID John Zurlo, Medical Staff Fellow, LCI/NIAID		
COOPERATING UNITS (if any) Harvey M. Friedman, University of Pennsylvania; Gary H. Cohen, University of Pennsylvania; Roselyn J. Eisenberg, University of Pennsylvania		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Clinical Immunology Section		
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS 1.75	PROFESSIONAL 1.5	OTHER 0.25
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Infection with herpes simplex type 1 (HSV-1) induces expression of receptors for C3b on mammalian cells previously devoid of such receptors. We have shown that the isolated glycoprotein responsible for this activity (gC) is a potent modulator of complement-mediated cytolysis in-vitro, acting by at least two mechanisms. We have demonstrated that herpes simplex type 2 (HSV-2) also induces expression of a C3b binding protein which corresponds to HSV-1 gC, but has different regulatory properties vis-a-vis the complement cascade. Binding requirements relating to glycosylation have been explored in a purified protein system for both gC-1 and gC-2. Transient expression systems in mammalian cells and a stably transfected murine cell line which expresses gC-1 under the control of an inducible promoter have been developed in the laboratories of Drs. Friedman and Cohen, and these systems are in use to study the binding site for C3b and also to explore the biologic consequences of the expression of gC vis-a-vis complement dependent host defense processes. Ongoing studies of parainfluenza virus type 3 have shown that certain IgM monoclonal antibodies to the hemagglutinin-neuraminidase protein can produce neutralization in the presence of an active complement cascade. This activity is dependent in toto on viral lysis. The antibodies do not function by initiating complement activation. The virus itself is a spontaneous activator of the classical pathway, an event which proceeds through specific binding of C1 from non-immune sera or in the purified state. Active monoclonals induce no overall enhancement of complement fragment uptake through C3, although late component binding is somewhat enhanced. This latter finding does not clearly distinguish monoclonals which produce synergistic complement-dependent neutralization from those that do not, however. Current studies are directed at elucidating how active monoclonals enhance the efficacy of the complement late components and the potential biologic implications of the C1 binding interaction.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00470-03 LCI															
PERIOD COVERED October 1, 1987 to September 30, 1988																	
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Sporadic Neurasthenia Associated With Epstein-Barr Virus																	
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 40%;">S. E. Straus</td> <td style="width: 45%;">Senior Investigator, LCI, NIAID</td> </tr> <tr> <td>OTHER:</td> <td>J. Dale</td> <td>Clinical Research Nurse, LCI, NIAID</td> </tr> <tr> <td></td> <td>J. Ostrove</td> <td>Senior Staff Fellow, LCI, NIAID</td> </tr> <tr> <td></td> <td>K. Croen</td> <td>Medical Staff Fellow, LCI, NIAID</td> </tr> <tr> <td></td> <td>R. Holman</td> <td>Medical Staff Fellow, LCI, NIAID</td> </tr> </table>			PI:	S. E. Straus	Senior Investigator, LCI, NIAID	OTHER:	J. Dale	Clinical Research Nurse, LCI, NIAID		J. Ostrove	Senior Staff Fellow, LCI, NIAID		K. Croen	Medical Staff Fellow, LCI, NIAID		R. Holman	Medical Staff Fellow, LCI, NIAID
PI:	S. E. Straus	Senior Investigator, LCI, NIAID															
OTHER:	J. Dale	Clinical Research Nurse, LCI, NIAID															
	J. Ostrove	Senior Staff Fellow, LCI, NIAID															
	K. Croen	Medical Staff Fellow, LCI, NIAID															
	R. Holman	Medical Staff Fellow, LCI, NIAID															
COOPERATING UNITS (if any) M. Kruesi (LCS, NIMH), J. Grafman (LMN, NINCDS), Z. Salahuddin, H. Streicher, R. Gallo (LTCC, NCI), M. Dalakas (NINCDS), N. Young (NHLBI)																	
LAB/BRANCH Laboratory of Clinical Investigation																	
SECTION Medical Virology Section																	
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892																	
TOTAL MAN-YEARS 2.5	PROFESSIONAL: 2.25	OTHER: .25															
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☒ (a1) Minors ☒ (a2) Interviews																	
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The goals of this project are to characterize severe chronic infections with Epstein-Barr virus, to ascertain whether Epstein-Barr virus, the new human B lymphotropic herpes virus, or other infectious agents can initiate a syndrome of chronic fatigue, and to characterize multiple aspects of the chronic fatigue syndrome. To date this research project has involved nearly 150 patients. Included are six patients with severe chronic Epstein-Barr virus infections. During the past year we began to use in-situ hybridization to study EBV gene expression in the tissues of these severely ill patients. All of the patients were proven to have increased levels of EBV RNA. On this basis and on the basis of other historical, clinical and serologic features of these patients, we have proposed criteria by which severe chronic EBV infection can be diagnosed. We initiated a detailed survey of our patient population for evidence of prior HBLV infection. Initial controlled seroprevalence studies showed seropositivity to be higher in patients with chronic fatigue. Detailed neurologic, neuroendocrinologic, and neuropsychologic studies were conducted in selected patients with chronic fatigue. To date we have found no reproducible abnormalities in the chronic fatigue patients but for a high prevalence of depression and other neurosis. Having shown acyclovir to not benefit patients with the chronic fatigue syndrome, we conducted a series of open therapeutic trials involving doxepin, H1 blockers, and doxycycline. None of these have yielded impressive benefits. Trials involving other classes of anti-depressant drugs are now being considered. In collaboration with investigators elsewhere we have formulated research criteria for the chronic fatigue.																	

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 201 AI 00481-03 LCI
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Receptors and Transduction Mechanisms in Human Phagocytes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation) PI: Harry L. Malech, M.D. Head, Bacterial Dis. Sect. LCI/NIAID Others: John I. Gallin, M.D. Scientific Director, IRP, NIAID Daniel Rotrosen, M.D. Senior Staff Fellow LCI/NIAID Philip Murphy, M.D. Medical Staff Fellow LCI/NIAID Thomas Leto, Ph.D. Senior Staff Fellow LCI/NIAID Hiroyuki Nunoi, M.D., Ph.D., Visiting Associate LCI/NIAID Michael Kleinberg, M.D., Ph.D., Med. Staff Fellow LCI/NIAID		
COOPERATING UNITS (if any) Coop. Units: Allen Speigel, MDB, NIADDKD; John Albert, Molec. Virol. Immunol. Div., Georgetown Univ., Rockville, MD;		
LAB-BRANCH Laboratory of Clinical Investigation		
SECTION Bacterial Diseases Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS 4.7	PROFESSIONAL 3.7	OTHER 1.0
CHECK APPROPRIATE BOXES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☒ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Human neutrophils</u> and monocytes are attracted to sites of inflammation, engulf microorganisms and secrete granule contents and toxic oxygen products. The purpose of this project is to define the biochemical structure and function of surface receptors, <u>transduction proteins</u> and effector systems involved in phagocyte responses to inflammation. Studies focus upon: (i) the receptor for bacterially derived formyl peptides, the formyl peptide chemotactic receptor (FPCR); (ii) pertussis toxin inhibitable GTP binding <u>regulatory proteins</u> (Gi) in myeloid cells required for FPCR and other receptor mediated cellular responses; (iii) cytosolic transducer proteins and the membrane <u>cytochrome b₅₅₈</u> (CYTO b) mediating <u>superoxide production</u>; (iv) <u>cytoskeleton</u> effector proteins involved in secretion. We show that both FPCR and Gi are present and functionally coupled in an intracellular pool in neutrophils. About 30% of neutrophil Gi is intracellular and this pool contains a higher ratio of Gi3 relative to Gi2 than that seen in plasma membrane. Using an antibody we developed to the C-terminus of the large subunit of CYTO b, we showed that CYTO b large subunit is transmembrane, that the C-terminus is cytoplasmic and plays an important functional role in superoxide production, and that >90% of CYTO b is intracellular in resting neutrophils and translocated to the surface with activation. We show that a 47 kDa and 65 kDa cytoplasmic proteins are essential for the transduction events in neutrophils leading to superoxide production and are defective in chronic granulomatous disease. Glycosylation of CYTO b differs in neutrophils and eosinophils. We find that the cytoskeletal protein, <u>fodrin</u>, is the major calmodulin binding protein in neutrophils.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00482-03 LCI
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Endothelial Cells in Inflammation		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Daniel Rotrosen, M.D. Medical Staff Fellow LCI/NIAID Others: John I. Gallin, M.D. Scientific Director, IRP, NIAID Harry L. Malech, M.D. Head, Bacterial Dis. Sect. LCI/NIAID		
COOPERATING UNITS (if any) Kevin Foskett, Ph.D., Department of Physiology, Armed Forces Radiobiology Research Institute, Bethesda, MD		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Bacterial Diseases Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: ---
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☒ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) (PROJECT TERMINATED) The vascular <u>endothelial cell</u> is uniquely situated to play an active role in the transduction of pro-inflammatory signals from the interstitial tissues to circulating <u>neutrophils</u>. The goals of our principal projects are (i) to study the role of endothelial cell receptor mediated transduction events in response to humoral inflammatory mediators; (ii) to examine the role of the endothelial cell in transduction of <u>chemoattractant</u> signals from interstitial sites to circulating <u>leukocytes</u>; and (iii) to examine the interaction of endothelial cells with neutrophils in the inflammatory response; and (iv) to examine the interaction of endothelial cells with pathogenic microorganisms. We demonstrated that endothelial cells bind formyl peptide chemoattractants, and release them intact. The characteristics of the binding site on endothelial cells differs in affinity and binding specificity from that found on phagocytic cells. The endothelial cell may direct <u>transport</u> of chemoattractant peptides from interstitial sites to the vascular lumen. In other studies we examined the interaction between <u>fungal hyphae</u> and endothelial cells, documenting a protective effect of neutrophils on the endothelial cell damage by fungi.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00495-02 LCI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT 80 characters or less Title must fit on one line between the borders)

Biochemical Events in Mast Cell Secretion

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID
Others:	Robert J. Hohman, Ph.D.	Expert	LCI/NIAID
	Juan L. Rodriguez, M.D.	NRSA Fellow	LCI/NIAID
	Raynaldo A. Martin	Biologist	LCI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

2.1

PROFESSIONAL

1.1

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|---|
| ☐ (a) Human subjects | ☐ (b) Human tissues | ☒ (c) Neither |
| ☐ (a1) Minors | | |
| ☐ (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Rat basophilic leukemia (RBL) cells are being employed as a model system to explore the biochemical events involved in IgE receptor mediated exocytosis in mast cells. A major obstacle to studying the molecular events in mast cell activation is the inability to introduce compounds of interest into the cytoplasm in such a way as to preserve intracellular structures and the intracellular concentration of macromolecules. We have developed a method for permeabilizing RBL cells to small molecules ($M_r < 1000$) using alpha toxin from *S. aureus*. The permeabilized cells respond to IgE receptor aggregation by activation of phospholipase C (PLC), resulting in hydrolysis of membrane phosphatidylinositol (PI), and release of histamine from secretory granules.

We are characterizing IgE receptor mediated PI hydrolysis and exocytosis in permeabilized cell preparations. Both responses are independent on extracellular Ca^{2+} in permeabilized cells; however, intact cells require millimolar Ca^{2+} for both events. Both PI breakdown and exocytosis are inhibited by diisopropylfluorophosphate (DFP), an inhibitor of serine esterases. Using the permeabilized cells, effectors of the various steps involved in IgE receptor mediated exocytosis can be directly introduced into RBL cells. The purpose of this project is to identify events occurring between IgE receptor aggregation and PI hydrolysis, and to examine what role (if any) PI hydrolysis plays in exocytosis.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00496-02 LCI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Immunodeficiency Virus (HIV) and Herpesviruses.

Interactions between the Human

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. M. Ostrove

Senior Staff Fellow, LCI, NIAID

Other: K. Croen

Medical Staff Fellow, LCI, NIAID

A. Rabson

Senior Staff Fellow, LMM, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.25

PROFESSIONAL:

0.25

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The role of herpesviruses as a possible cofactor in the progression and pathogenesis of the acquired immune deficiency syndrome (AIDS) is being investigated. AIDS patients develop a wide variety of opportunistic infections and we have been studying the interactions of herpes simplex (HSV) and varicella-zoster (VZV) viruses with the human immunodeficiency virus (HIV) in controlled laboratory experiments. Recombinant clones containing the long terminal repeat (LTR) sequences from the HIV were linked to the indicator gene chloramphenicol acetyltransferase (CAT) and CAT activity was used as a measure of stimulation of the HIV. Infection of cells harboring the HIV-LTR-CAT with herpes simplex virus types 1 or 2 (HSV) or varicella-zoster virus led to stimulation of CAT activity. Two HSV genes, ICP4 and ICP0 and two VZV genes, the products of open reading frames 4 and 62 were shown to be responsible for this effect. The stimulation seen with these HSV and VZV genes differed depending on which cell type was used implying that cellular proteins were playing a major role in the HIV activation seen by herpesviruses. Analysis of deletion mutants within the HIV-LTR revealed that the regions of the LTR required for HSV activation maps to the SP1 and NF-kB binding sequences. The experiments showing HSV activation were extended by the use of an infectious clone of HIV whose replication can be stimulated by HSV infection as well as by various cloned HSV genes. We have shown that in HeLa cells, HSV infection stimulates the release of active NF-kB that is required for HIV LTR directed transcription. We are now in the process of determining if this is the mechanism by which HSV activates HIV gene expression.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00513-01 LCI

PERIOD COVERED:
October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Biology of Mast Cell Growth and Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator (Name, title, laboratory, and institute affiliation))

PI: Dean D. Metcalfe, M.D. Head, Mast Cell Physiology Section LCI/NIAID
Others: Arnold S. Kirshenbaum, M.D. NRSA LCI/NIAID
Beth Friedman, M.D. Medical Staff Fellow LCI/NIAID
Helen Thompson, Ph.D. Visiting Fellow LCI/NIAID

COOPERATING UNITS (if any)

The University of Washington (C.W. Henderson and S. Klebanoff)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mast Cell Physiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

3.50

PROFESSIONAL

3.00

OTHER:

0.50

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Human bone marrow under the influence of human IL-3 and in suspension culture gives rise to 10 to 30% basophils. When human bone marrow is cultured on the surface of agarose in the presence of IL-3, both basophils and mast cells can be identified that reach maximum numbers by 2 to 3 weeks.

The IL-3 dependent growth of human basophils and mouse mast cells in suspension culture is inhibited by granulocyte-macrophage colony stimulating factor.

The IL-3 dependent growth of human basophils and mast cells in culture is not influenced by the removal of cells bearing T cell, B cell, or macrophage markers.

Human lung mast cells release both heparin and chondroitin sulfate E. Mast cell proteoglycans are susceptible to cleavage by both hydroxyl radicals and hypochlorous acid.

Bi-directional phenotypic changes in mast cells can be identified in vivo. This demonstrates that local factors also play an important role in the final stages of mast cell differentiation.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00514-01 LCI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Diagnosis and Treatment of Adverse Reactions to Foods and Additives

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Dean D. Metcalfe, M.D. Head, Mast Cell Physiology Section LCI/NIAID

COOPERATING UNITS (if any)

Indian Institute of Science, Bangalore, India (P.V. Subba Rao)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mast Cell Physiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.50

PROFESSIONAL:

0.25

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

On a collaborative project, with Dr. P.V. Subba Rao at the Indian Institute of Science, we have isolated and characterized three antigens from shrimp. These three substances appear to account for the majority of allergenic material. Two are glycoproteins, and a third is tRNA. The characterization of these antigens leads the way to the production of improved diagnostic and therapeutic reagents for the management of shrimp sensitivity.

Six normal subjects and twelve patients with suspected sensitivity to aspartame have participated in a NIAID-CRS approved blinded challenge protocol. One individual with a history of adverse reactions developed several hives during single- but not double-blind challenge. Plasma histamine levels remained normal in all cases.

Two normal subjects, three asthmatics, and one individual with a history of hives from MSG have participated in a NIAID-CRS approved protocol for MSG sensitivity. No adverse responses were noted to MSG, and no changes occurred in plasma histamine levels.

Measurable amounts of histamine were found within a number of foods. The amount of histamine appears to be insufficient to induce a clinical response by itself alone, but suggests the possibility that histamine may potentiate reactions to other substances within foods.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00521-01 LCI
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Effect of Cytokines in Host Defense and Inflammation		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: John I. Gallin, M.D. Director, IRP, NIAID Others: Joan M.G. Sechler LCI/NIAID Harry L. Malech, M.D. Head, Bacterial Dis. Sec. LCI/NIAID Phil Murphy, M.D. Medical Staff Fellow LCI/NIAID		
COOPERATING UNITS (if any) Thomas Nutman, M.D., LPD/NIAID; Chris King, M.D., LPD/NIAID; Clifford Lane, M.D., LIR/NIAID; Anthony S. Fauci, M.D. LIR/NIAID		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Bacterial Diseases Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.8	0.8	1.0
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☒ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Studies have been initiated to explore the importance of <u>cytokines</u> in phagocyte function. Studies of <u>phagocytes</u> from patients with AIDS who were receiving <u>interferon gamma</u> or interleukin-2 indicated that patients receiving interferon gamma had markedly fewer bacterial infections than those receiving interleukin-2. We also studied the effect of interferon gamma on <u>neutrophils</u> and <u>monocytes</u> from patients with <u>chronic granulomatous diseases of childhood</u> (CGD). Interferon gamma corrected the abnormal <u>oxidative metabolism</u> in the phagocytes of many CGD patients <u>in vitro</u> and <u>in vivo</u> the drug was shown to reconstitute the defect as well. On other studies the effect of interferon gamma in the <u>Hyperimmunoglobulin E and Recurrent Infections (Job's) Syndrome</u> indicate that <u>in vitro</u> and <u>in vivo</u> interferon gamma down regulates B cell synthesis of <u>IgE</u> while <u>in vivo</u> interferon gamma lowers the elevated <u>IgE</u> in this syndrome towards normal. Interferon gamma was shown to cause monocyte <u>polykaryon</u> formation, in an <u>in vitro</u> model of <u>granuloma formation</u>, through a cascade of events involving <u>interleukin-1</u> and <u>tumor necrosis factor</u>. The latter effects could be blocked <u>in vitro</u> by use of <u>corticosteroids</u>. In other studies of the growth of mononuclear phagocytes, a technique was determined for developing <u>human macrophage cell lines</u> without transformation. Studies of the effect of interferon gamma on monocyte polykaryon formation demonstrated that human macrophages produce copious amounts of <u>CSF-1</u> and this may relate to an additional observation that under appropriate conditions <u>human monocyte replication</u> occurs <u>in vitro</u> when using "conditioned" growth media, rich in CSF-1.		

LABORATORY OF IMMUNOGENETICS
1988 Annual Report
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ANNUAL REPORT
LABORATORY OF IMMUNOGENETICS
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
October 1, 1987 to September 30, 1988

RESEARCH PROGRESS

The laboratory of Immunogenetics investigates the multigene families that are involved in the control of immune function. Research emphasizes the structure and function of these genes and their products as well as mechanisms for regulation of these genes. The major histocompatibility complex, the T cell antigen receptor and the immunoglobulin gene complexes of several different species have been studied. Most recent investigations concern studies of disease models involving disfunction of the immune system, as well as studies of the role of MHC antigens and T cell receptor molecules in antigen presentation. A wide range of techniques at the molecular, serological and functional levels are used in these investigations.

NORMAL AND PATHOLOGIC IMMUNE RESPONSE IN THE LABORATORY RABBIT

The rabbit has in the past proven to be an excellent model for various diseases, including lung damage, herpes infection, diseases of the eye, syphilis, and it has been shown that the rabbit is a permissive host for HTLV-1 infection. Recent investigations concern a disease model for crescentic glomerulonephritis in the rabbit and a study of HTLV-1 and HIV infection in this species has begun. Evidence from both in vitro and in vivo studies indicates that rabbits are susceptible to infection with HIV-1 the virus that is the causative agent of the disease AIDS. Studies are under way to characterize the parameters of infection in rabbit cells and in the animal itself.

Viral Infection of Rabbit Cells. It has been found that rabbit T cell and macrophage lines can be infected in vitro with HIV (Kulaga). The infected cells product reverse transcriptase RNA viral transcripts and are positive for viral proteins both by immunofluorescence analysis and by western blots using AIDS patients serum. Although transformed rabbit T cell and macrophage lines could be infected by HIV similar attempts to infect fibroblast lines and normal peripheral blood lymphocytes (PBL) were not successful. In light of these results, it was attempted to carry out in vivo infection of rabbits with HIV. Because reported attempts to infect rabbits with HIV alone had not been successful, infections were carried out using a human T cell line infected with HIV as the source of virus. In addition, it was attempted to optimize the possibility of infection using rabbits that had previously seroconverted to the virus HTLV-1. It was found that all rabbits injected with the infected human T cell line were positive for HIV after several weeks. Antibody to HIV-1 proteins could be detected in the rabbit serum and the virus could be cultured from peripheral blood cells taken from the infected animals. It was rather surprising that the group of rabbits that had not been previously infected with HTLV-1 were likewise infected with HIV. This was not expected because of the in vitro results indicating that rabbit PBL's are not infected by HIV under normal circumstances.

The infected animals showed sporadic signs of illness when examined by gross observation and by chemical and hematologic analysis of blood. The organs from necropsy of singly or doubly infected animals showed splenic enlargement with hyperplasia evident upon histologic examination. It was also shown that the lungs of infected animals were infiltrated with lymphocytes.

Little is known about subpopulations of rabbit T-lymphocytes. It is not known for example if there are receptors on helper and killer T cells which are homologous to CD4 and CD8 in man. Efforts are underway to determine whether these markers are present on rabbit cells. These studies involve use of synthetic oligonucleotide probes the structures of which are based on consensus sequences obtained from human, rat and mouse CD4 and CD8 proteins. These probes will be used to screen cDNA libraries prepared from RNA rabbit lymphoid tissue and to monitor RNA production in different rabbit T cell lines (Gordon).

HTLV-1 Infection in Rabbits. It has been known for several years that injection of rabbits with human T cell lines infected with the human T cell leukemia virus, HTLV-1 causes infection in the rabbit. Recently it has been shown that there are disease symptoms that accompany this infection. Our studies have involved infection of rabbits with HTLV-1 and derivation of T cell lines from PBL from these animals. In addition, the choroid plexus (CP) the brain of an infected rabbit was placed in culture. A cell line has been derived from the CP and it appears to be transformed (Truckenmiller). It is presently being examined for the presence of HTLV-1 genome in the cellular DNA. This is particularly exciting because HTLV is known to cause a disease called "Tropical Spastic Paraparesis" in man. This disease affects the central nervous system. Use of the polymerase chain reaction has facilitated the search for HTLV-1 gene products in potentially infected cells. Rabbits with HTLV-1 will be carefully studied to determine whether hallmarks of the adult T cell leukemia, such as overexpression of IL2 receptors on T lymphocytes are seen in this animal model.

Characterization of Rabbit MHC Antigens. The rabbit MHC locus designated RLA encodes class I and class II molecules similar to those encoded in the human and murine system. However, the rabbit class I family is smaller than that seen for mouse or man, thus facilitating studies on these molecules. In the past year a T cell specific class I antigen has been characterized according to its structure and to its pattern of expression (Rebiere). The transcript for this T cell specific class I gene was found only in cymes, appendix and spleen, even when the sensitive nucleus protection assay was used to search for RNA corresponding to this gene. A search for a sequences involved in control of the expression of class I genes is underway. These studies will involve searching at the 5' region around the promoter for sequences that are involved in control of gene expression. This can be carried out by cloning fragments of rabbit class I genes next to CAT genes and transfecting appropriate cells with these hybrid constructs (Lubin). Several regions in the 5' flanking section of an expressed rabbit class I gene have been found, that are homologous to regulatory sequences found in mouse and man.

Studies on class II MHC antigens in rabbit continue to indicate a close similarity between the class II families in rabbit and in man. A recent complete of RLA-DR has been completed and sequence comparisons showed highest homology to the human HLA-DR gene. Probes derived from both class I and class II MHC genes in the rabbit have been used in an attempt to establish typing procedures for this species. Because of the lack of suitable serologic reagents, it has been necessary to use molecular typing procedures in order to obtain RLA typing in the rabbit. In collaboration with Dr. Katherine Knight, it has been shown that a small number of probes and enzymes digests may be used to establish haplotype identification in the rabbit.

Experimental Crescentic Glomerulonephritis (CGN). It has been shown that the rabbit is a suitable subject for studies of CGN. This disease may be induced by injection of immunoglobulin and antibodies directed toward rabbit basement membrane. In collaboration with workers at the University of Michigan, we have shown that cells that infiltrate the kidney in the course of this condition are T cells and these become activated as the disease progresses. It is our intention to characterize these cells by their cell surface markers and by the

specificity of their antigen receptors (Lee). These studies will be coordinated with other investigations of T cell sub-populations in the rabbit.

Studies of Human T cell Receptor Genes. In order to investigate the genetic control of human immune responses, the inheritance of several genes known to play important roles in a variety of immune responses (TCR) genes have been examined by Southern blot analyses using specific DNA probes to identify restriction fragment length polymorphism of TCR genes detected by RFLP in that few restriction enzymes reveal polymorphism with a given probe and when polymorphism is observed there are few allelic forms of each marker. However, there is considerable polymorphism in the combination of markers inherited together in both TCR alpha and TCR beta haplotypes. Recent evidence for recombination within the TCR alpha gene complex has been obtained in family studies (Robinson). Population studies suggest that recombination has been frequent in this gene complex. These observations have an important impact upon studies to determine whether genes are associated with disease susceptibility. TCR and disease association studies will most informatively be performed in families showing a predisposition to a particular disease.

There is compelling evidence that susceptibility to Multiple Sclerosis is inherited and that T lymphocytes play a role in the pathogenesis of the disease. HLA and TCR haplotypes were determined in sibships with two or more members concordant for MS. Of the 16 families studied to date, 4 affected sib-pairs shared 0 haplotypes, 11 pairs shared 1 haplotype and 1 pair shared 0 haplotype. In addition to the preponderance of shared haplotypes by patients there was also a TCR beta RFLP which was present at an increased frequency in unrelated MS patients. Correlation between RFLP and sequence differences in TCR beta V region coding sequences was examined by amplification of genomic DNA using the Polymerase chain reaction (PCR), cloning into m13, and DNA sequence analysis (Grier).

GENE CONTROL EXPRESSION

Investigations of gene regulation in B lymphocytes continue to focus on two model systems: The human kappa light chain immunoglobulin gene and the human J chain (Flanagan). The expression of the kappa immunoglobulin gene is regulated by a transcriptional enhancer located in the J-C intron. Two domains within the enhancer that bind nuclear proteins have been studied. A protein which binds to the 3' domain (at the kappa E2 site) has been further purified (now approximately 5000-fold) and a molecular weight estimate has been obtained by UF-crosslinking/SDS-PAGE analysis. In investigations of proteins binding to the 5' domain of the enhancer, two forms of binding activity at the kappa b motif were detected by an exonuclease protection assay of nuclear extracts; NF kappa B1 and NF kappa B2. The latter activity is widely distributed, even in cells that do not transcribe kappa. NF kappa B1 activity, however, seems to be confined to cells in which the kappa gene is transcribed; it may be the functional form that mediates enhancer activity. In nuclear extracts from several sources in which the enhancer is inactive, NF kappa B1 binding activity could be induced by *in vitro* treatment with guanidine at millimolar concentrations (Recker). It is possible that the induction observed *in vitro* with guanidine may occur by a mechanism similar to the *in vivo* induction of NF kappa B reported in response to lymphokines. The H chain promoter region has been analyzed by transfecting into a myeloma a series of constructs in which the promoter--with various deletions--is linked to a chloramphenicol acetyl transferase (CAT) gene. Deletions in three regions of the promoter have been found to affect (CAT) transcription in a positive or negative way. *In vitro* investigations of nuclear extract proteins capable of binding in the promoter region have detected several binding sites, at least one of which appears to coincide with functionally important sequence (as demonstrated by the deletion analysis).

MOLECULAR AND FUNCTIONAL ANALYSIS OF HLA CLASS II ANTIGENS

An immune response occurs only if a T lymphocyte has been able to recognize a foreign antigen in association with a self molecule encoded in the major histocompatibility complex (MHC). MHC molecules expressed on antigen-presenting cells are the polymorphic class II antigens. Most foreign antigens must be processed in order to bind to MHC molecules. How the processed peptides are generated and where they interact with MHC molecules is largely unknown. Experiments in progress aim to define the function of the different human class II antigens in their interaction with T cells and to learn the requirements for class II-restricted processing and presentation of viral antigens. Three isotopic class II antigens are expressed in man: HLA-DR, -DP, and DQ. Two additional transcribed genes have been identified by cDNA cloning and sequencing. DN alpha (Rosen, Bronsen) and DO beta (Long). The interaction between a class II MHC molecule and a specific T cell is complex because it involves (i) an immunogenic peptide, (ii) and antigen-specific T cell receptor (TCR), and (iii) the CD4 molecule expressed on the T cell. In order to dissect these interactions an assay has been developed that measures a functional CD4 class II interaction in the absence of a TCR-class II interaction (Long, Sekaly, Karp). Using this assay, it has been demonstrated that both HLA-DR and DP antigens can interact with CD4 and that the site on CD4 involved in this interaction is distinct from that to which HIV binds.

The cytotoxic T lymphocyte (CTL) response to measles virus is primarily MHC class II-restricted. Transfected cells expressing HLA-DR antigen and cytoplasmic viral antigens were efficiently lysed by measles virus - specific CTL's, demonstrating that endogenously synthesized cytoplasmic proteins can be presented by class II MHC antigens (Long, Jacobson). In addition studies on the HLA-DN gene have indicated that more than one transcript is made. A novel type of alternative splicing has been found in DN alpha (Rosen-Bronson). The alternatively spliced RNA encodes a short protein totally unrelated to the class II DN alpha chain, except for a shared signal sequence.

Analysis of Human Killer T cells. Peripheral blood lymphocytes grown in the presence of high doses of interleukin-2 (IL-2) acquire the ability to lyse tumor cells. This cytolytic activity is not restricted by antigens encoded in the major histocompatibility complex (MHC) and is observed with a variety of both autologous and allogeneic tumors. This activity has been called lymphokine-activated killing (LAK) and has been used in clinical treatment of tumors. The origin of cells with LAK activity and the mechanism by which they recognize and kill their targets are unknown.

More than 20 clones isolated from human peripheral blood were analyzed for their ability to lyse fresh tumor cells and for their cell-surface phenotype. Most of the clones with LAK activity has the CD3⁺ CD8⁺ CD16⁺ phenotype. Populations of cells with this phenotype were purified by a combination of antibody-mediated complement killing and cell sorting with the fluorescent activated cell sorter. RNA extracted from clones and enriched populations of cells with LAK activity was analyzed by hybridization with DNA probes for the presence of transcripts derived from the CD3 and TCR genes (Biassoni). Absence of mature transcripts from the CD3 gamma, CD3 delta, TCR beta and TCR gamma genes showed that recognition of tumor cells was not mediated by the known CD3-TCR alpha-beta or gamma-delta complexes. Interestingly, all CD3⁺ cells with LAK activity expressed the CD3 epsilon and TCR delta genes. This finding identified these cells as members of an early stage in the T cell lineage.

Expression of genes encoding various lymphokines in activated CD3⁺ CD4⁺ CD8⁺ CD16⁺ cells was measured. The pattern of lymphokine expression was also consistent with an early stage of T cell maturation. These studies identified for the first time that cells with LAK

activity belong in the T cell lineage and, furthermore, that they represent the earliest known stage in T cell differentiation.

LABORATORY OF IMMUNOGENETICS

Annual Report

October 1, 1987 to September 30, 1988

HONORS AND AWARDS

In the past year Dr. Kindt has presented invited seminars on laboratory data at Emory University, Amherst College, the University of Connecticut Health Center, Case Western Reserve University, Georgetown University, Oxford University, the Imperial Cancer Research Institute and at the Pasteur Institute. Seminars were also presented at Merck and Company and at Hybridtech Incorporated. Results of laboratory work were presented at the FASEB meeting in Las Vegas, at the International Workshop on H2 and HLA Gene Cloning and at the International AIDS meeting in Stockholm. Dr. Kindt continues to serve as Deputy Editor of the Journal of Immunology and on the Editorial Advisory Board of the Journal of Experimental Medicine. He has recently been invited to serve as Editor for a new journal Current Opinion in Immunology and has been invited to serve as Regional Editor for the Annals of Institute Pasteur. Dr. Kindt currently serves on the Scientific Advisory Board of Oncor Incorporated and Southern Biotech Incorporated. He is a member of a review panel for the Multiple Sclerosis Society and holds a position as Adjunct Professor at the Georgetown University School of Medicine. Dr. Mary Ann Robinson has served on the program committee for the ASHI meeting and chaired a session and presented data at this meeting. She has presented seminars on her data at Howard University. In October she was an invited guest of the Government of the Peoples Republic of China, presented a seminar and held workshops on molecular genetics in Shanghai. Dr. Eric Long participated in the International Workshop on the Molecular Basis of Antigen Presentation by MHC Molecules in Paris, has presented a paper at the symposium Gene Regulation in the Developing Immune System at the NIH and participated in the UCLA Symposium Cell Biology of Virus entry, Replication and Pathogenesis in Taos, New Mexico. He was also an invited speaker at the Pittsburgh Cancer Institute this year. Drs. Long and Kindt were co-organizers of the Sixth National HLA H2 Cloning Workshop that was held in Arlie, Virginia in May 1988 and both chaired sessions at this meeting.

Administrative Report

During the past year, several postdoctoral fellows have joined the lab. These are Dr. Emmy Truckenmiller from Georgetown University and Dr. Alice Grier also from Georgetown University who have joined the Immunogenetics Research Section. Dr. Dolores Jaraquemada from the University of London has joined the Molecular Immunology Section and Dr. Kevin Lee from Temple University was award an NRC Fellowship to work with Dr. Kindt in the Immunogenetics Research Section. Departures from the lab have included Dr. Fatia Mami who has accepted a permanent position at the Institute Gustave Roussy outside of Paris and Dr. Sandy Rosen-Bronson who was named Assistant Professor at Georgetown University. Dr. Henrietta Kulaga was appointed to a position at the National Institutes of Mental Health but will stay for this year and serve as a Special Volunteer in the Immunogenetics Research Section to continue work that she has begun. Dr. James Flanagan will join the Institute for Child Health and Development to continue his fellowship training.

LABORATORY OF IMMUNOGENETICS
Annual Report
October 1, 1987 to September 30, 1988

LAY LANGUAGE SUMMARY

Significant Accomplishments

Rabbit cell lines have been infected in culture with Human Immunodeficiency Virus-1 (HIV-1) the causative agent of human AIDS. Recently evidence has been obtained for in vivo infection of rabbits with this virus and studies are directed toward definition of an animal model for HIV-1 infection. Such a disease model would greatly facilitate development of vaccines and testing of therapeutic agents for this disease. It would furthermore allow detailed studies of the mode of transmission of HIV-1 and allow examination of the natural course of this disease under controlled experimental circumstances.

New Initiatives

The retrovirus HTLV-1 has been shown to be the causative agent for adult T cell leukemia (ATL) and is strongly implicated in a neurological disorder called tropical spastic paraparesis. It has been shown by others that this virus causes infection in rabbits. Recent efforts in our laboratory have been directed toward molecular definition of the disease caused by HTLV-1. Cell lines have been derived from the peripheral blood of rabbits infected with HTLV-1 and placed in culture. These cells are under study to determine what markers are expressed in comparison to normal T lymphocytes taken from uninfected rabbits. In addition, studies of brain cells from infected rabbits indicate that certain cells (choroid plexus) can be transformed with HTLV-1. These studies could provide evidence concerning the mechanisms by which HTLV-1 causes disease in man and could lead to preventative measures to combat these diseases. Although previously confined to Southern Japan and certain Caribbean countries, HTLV-1 disease is becoming more common in the United States, particularly among intravenous drug users.

Preliminary studies of T cell receptor genes in human families having more than one member of with Multiple Sclerosis have indicated a possible linkage of a gene in the T cell receptor complex to susceptibility to Multiple Sclerosis. Like certain other autoimmune diseases susceptibility to MS is increased in persons having certain HLA alleles. It is questioned whether a study of the HLA and TCR genes in patients may show an increase susceptibility in persons with certain combinations of alleles of these interactive gene families.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI-00166-11-LIG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Rabbit MHC Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)

PI: Thomas J. Kindt
 Others: M.C. Rebiere
 Ira Lubin

Chief
 Visiting Associate
 IRTA Fellow

LIG, NIAID
 LIG, NIAID
 LIG, NIAID

COOPERATING UNITS (if any)

Patrice Marche, Christian LeGuern, Institut Pasteur; K. Knight, University of Illinois, Chicago

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

3.2

PROFESSIONAL

2.2

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The rabbit MHC locus, designated RLA, encodes class I and class II molecules similar to those encoded in the human and murine systems. However, recent structural data on the MHC of the rabbit suggest that the RLA is considerably closer to that of man than to that of mouse. Protein structural and molecular genetic studies of the rabbit RLA genes have been conducted using a rabbit T-cell line, RL-5. The rabbit class I family contains only between 8 to 13 genes, as opposed to 18 and 36 in man and mouse respectively. Structural and sequencing data are now available for four cDNAs and three corresponding genes. One of them, 19-1, has been shown to encode the only class I protein expressed at the surface of the cells. Using probes derived from these genes, expression studies at the mRNA level have been performed using rabbit cells and normal rabbit tissues. The genes 19-1 and 5-2 are ubiquitously expressed. However, the third gene, 7-1, is transcribed only in T-cell lines and lymphoid tissue containing T-cells. Transfection experiments of the 7-1 gene to determine if 7-1 has the same specific pattern of expression in other cellular environments. The differential pattern of expression of the rabbit class I genes provide a useful system to study the mechanisms involved in the regulation of expression of these genes. Search for sequences and factors involved in the expression of the gene 19-1 are in progress by using CAT assays with the promoter region of this gene. DNA regions controlling the up regulation of the rabbit class I genes by several cytokines are also under investigation. Studies of the rabbit class II genes revealed that there are rabbit genes corresponding to every human class II genes found in the HLA-D region. Rabbit class II genes corresponding to the HLA -DR, -DP, -DQ, and -DZ α genes have been partially or fully sequenced. Genetic analysis based on RFLP using selected probes from class I and II genes have provided molecular data covering linkage of class I and class II subregions in RLA and new haplotypes were found in the typing an extended family.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI-00168-11-LIG
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cell Surface Markers of Rabbit Lymphocytes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Thomas J. Kindt Others: Mark Gordon Henriette Kulaga Kevin Lee F. Mami	Chief NRC Fellow Senior Staff Fellow NRC Fellow Visiting Fellow	LIG, NIAID LIG, NIAID LIG, NIAID
COOPERATING UNITS (if any) Mike Roy, Walter Reed Army Hospital		
LAB/BRANCH Immunogenetics Research Section		
SECTION Laboratory of Immunogenetics		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 2.4	PROFESSIONAL: 2.0	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither. ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This study aims to identify the cell surface markers of rabbit lymphocytes that correlate with functional subpopulations and to use these markers to identify changes in the subpopulations when the rabbit is infected with retroviruses or is subjected to autoimmune disease. Initial studies have concentrated upon the T-cell compartment and efforts are underway to identify the correlates of the human and murine markers for helper and cytolytic T-cells. Approaches to this problem have been through the use of monoclonal antibodies directed against cell surface antigens and be efforts directed toward molecular cloning of the genes for homologs of human CD4 and CD8. Antibodies have been used to monitor distribution of specific antigens in various cells and lymphoid organs. Further studies have used Western blot analyses to determine the size of antigens recognized by the antibodies. The molecular approach to the identification of CD4 like molecules in the rabbit has used screening of a cDNA library prepared from rabbit lymphoid cells with synthetic oligonucleotide probes the sequence of which is deduced from common regions of the human, mouse, and rat CD4 homologs.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI-00170-11-LIG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular and Functional Analysis of Human Class II Histocompatibility Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Eric O. Long	Visiting Fellow
Other:	Rafick Sekaly	Staff Fellow
	Sandra Rosen-Bronson	Medical Staff Fellow
	David Karp	Visiting Fellow
	Dolores Jaraquemada	

COOPERATING UNITS (if any)

Neuroimmunology Branch, NINCDS, NIH (S. Jacobson, H. McFarland); Clinical Research Institute of Montreal, Montreal, Canada (R. Sekaly)

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Molecular Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

3.75

PROFESSIONAL

2.75

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- | | | |
|--|--|--------------------------------------|
| ☒ (a) Human subjects | ☐ (b) Human tissues | ☐ (c) Neither |
| ☐ (a1) Minors | | |
| ☐ (a2) Interviews | | |

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

An immune response occurs only if a T lymphocyte has been able to recognize the foreign antigen in association with a self molecule encoded in the major histocompatibility complex (MHC). These molecules expressed on antigen-presenting cells are the polymorphic class II MHC antigens. Most foreign antigens must be processed in order to bind to MHC molecules. How the processed peptides are generated and where they interact with MHC molecules is largely unknown. The aim of this project is to define the function of the different human class II antigens in their interaction with T cells and the requirements for class II-restricted processing and presentation of viral antigens. Three isotypic class II antigens exist in man: HLA-DR, -D, and -DP. Two additional transcribed genes have been identified by cDNA cloning and sequencing: DNQ and DO β . A novel type of alternative splicing has been found in DN α . The alternatively spliced RNA encodes a short protein totally unrelated to the class II DN α chain, except for a shared signal sequence. The interaction between a class II MHC molecule and a specific T cell is complex because it involves (i) an immunogenic peptide, (ii) an antigen-specific T-cell receptor (TCR), and (iii) the CD4 molecule expressed on the T cell. In order to dissect these interactions an assay has been developed that measures a functional CD4-class II interaction in the absence of a TCR-class II interaction. Using this assay, it has been demonstrated that both HLA-DR and -DP antigens can interact with CD4 and that the site on CD4 involved in this interaction is probably distinct from that to which HIV binds.

The cytotoxic T lymphocyte (CTL) response to measles virus is primarily MHC class II-restricted. Transfected cells expressing HLA-DR antigen and cytoplasmic viral antigens were efficiently lysed by measles virus-specific CTLs, demonstrating that endogenously synthesized cytoplasmic proteins can be presented by class II MHC antigens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI-00171-11-LIG
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (40 characters or less. Title must fit on one line between the borders.) Infection of Rabbits with Human Immunodeficiency Virus I		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Thomas J. Kindt Other: Henrietta Kulaga	Chief Senior Staff Fellow	LIG, NIAID LIG, NIAID
COOPERATING UNITS (if any) T. M. Folks, Laboratory of Immunoregulation, NIAID; R. A. Rutledge, Laboratory of Molecular Microbiology, NIAID		
LAB/BRANCH Laboratory of Immunogenetics		
SECTION Immunogenetics Research Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 4.0	PROFESSIONAL: 1.5	OTHER: 2.3
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Injection of rabbits with human cells infected with human immunodeficiency virus I (HIV-1) has lead to seroconversion within six weeks and virus has been cultured from peripheral blood lymphocytes (PBL) from the injected animals. The use of rabbits for HIV-1 infection was prompted by our recent finding that rabbit T cell and macrophage lines infection <u>in vitro</u> with HIV produced reverse transcriptase, RNA viral transcripts and were positive for viral proteins, by immunofluorescence and by Western blots using AIDS patient sera. Evidence for <u>in vivo</u> infection was seen in rabbits that had been previously seroconverted with the virus HTLV-1 as well as in animals with no history of HTLV-1 infection. Sporadic signs of illness were seen in infected animals by gross observation and by chemical and hematologic analyses. Examination of organs from necropsy of single or doubly infected animals showed splenic enlargement with hyperplasia evident upon histologic examination which also revealed that lungs were infiltrated with lymphocytes. This animal model for HIV infection may be useful for testing vaccines and therapeutic drugs, for determining modes of HIV viral transmission and for following the natural course of HIV-1 infection in an experimental animal.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI-00173-11-LIG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Control of Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Edward E. Max
Other: James Flanagan
David Recker

Commissioned Officer
Medical Staff Fellow
Medical Staff Fellow

LIG, NIAID
LIG, NIAID
LIG, NIAID

COOPERATING UNITS (if any)

Dr. Suzanne Epstein, BOB, FDA; Dr. David Sachs, DCBD, NCI; Dr. Arifa Khan, LMM, NIAID; Dr. Arthur Krieg, ARB, NIAMS; Dr. Richard Asofsky, LMI, NIAID

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Molecular Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

4

PROFESSIONAL

3

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Investigations of gene regulation in B lymphocytes continue to focus on two model systems: the human κ light chain immunoglobulin gene and the human J chain gene. The expression of the κ immunoglobulin gene is regulated by a transcriptional enhancer located in the J-C intron. We have studied two domains within the enhancer that bind nuclear proteins. A protein which binds to the 3' domain (at the κ E2 site) has been further purified (now approximately 5000-fold) and a molecular weight estimate has been obtained by UF-crosslinking/SDS-PAGE analysis. In investigations of proteins binding to the 5' domain of the enhancer, we detected two forms of binding activity at the κ B motif by an exonuclease protection assay of nuclear extracts; we identify these as NF κ B1 and NF κ B2. The latter activity is widely distributed, even in cells that do not transcribe κ . NF κ B1 activity, however, seems to be confined to cells in which the κ gene is transcribed; it may be the functional form that mediates enhancer activity. In nuclear extracts from several sources in which the enhancer is inactive, NF κ B1 binding activity could be induced by *in vitro* treatment with guanidine at millimolar concentrations. It is possible that the induction observed *in vitro* with guanidine may occur by a mechanism similar to the *in vivo* induction of NF κ B reported in response to lymphokines. The J chain promoter region has been analyzed by transfecting into a myeloma a series of constructs in which the promoter--with various deletions--is linked to a chloramphenicol acetyl transferase (CAT) gene. Deletions in three regions of the promoter have been found to affect CAT transcription in a positive or negative way. *In vitro* investigations of nuclear extract proteins capable of binding in the promoter region have detected several binding sites, at least one of which appears to coincide with functionally important sequence (as demonstrated by the deletion analysis).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI-00180-10-LIG
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Properties of Transformed Rabbit Cell Lines		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Thomas J. Kindt Others: M. E. Truckenmiller K. A. Lee H. Kulaga	Chief IRTA Fellow NRC Fellow Senior Staff Fellow	LIG, NIAID LIG, NIAID
COOPERATING UNITS (if any) B. Potts, LMM, NIAID; Roger Wiggins, Univ. of Michigan		
LAB/BRANCH Laboratory of Immunogenetics		
SECTION Immunogenetics Research Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 2.7	PROFESSIONAL: 2.0	OTHER: 0.7
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) These studies involve derivation of transformed cell lines from the rabbit. Recent attempts have utilized the retrovirus HTLV-1 which is the causative agent in the human adult T-cell leukemia and which has been implicated in the disease tropical spastic paraparesis. Lines have been derived from the peripheral blood of rabbits infected with HTLV-1 and preliminary data show that these are T-cell lines. These cells contain DNA corresponding to HTLV-1; the precise pattern of integration and the extent of the proviral genome in the lines will be measured. Choroid plexus was taken at necropsy from a rabbit that had been infected with HTLV-1 for about 1 year and an adherent cell line has grown from this tissue. Rabbit lines that are transformed with HTLV-1 appear to be highly susceptible to infection with HIV-1. The possible reasons for this are being investigated. Collaborative studies with nephrologists at U. Mich. are aimed at derivation of cell lines from kidneys of rabbits with experimentally induced crescentic glomerulonephritis (CGN). It has been shown in preliminary studies that the cells infiltrating the kidney in the course of this condition are T-cells and that they become activated as the disease progresses. It is our intention to characterize these cells both by their cell surface markers and by the specificity of their antigen receptors.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI-00389-05-LIG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (do not exceed 80 characters or less. The title should not include the words "Notice of Intramural Research Project")

Molecular Genotype Analyses of HLA and TCR genes in Human Families

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)

PI: Mary Ann Robinson

Senior Staff Fellow

LIG, NIAID

Others: Alice Grier

IRTA

COOPERATING UNITS (if any)

D. Bernard Amos, Duke University, S. Hauser, Massachusetts General Hospital; D. Posnett, Cornell University Medical Center

LABORATORY

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

2.6

PROFESSIONAL

2

OTHER

0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In order to investigate the genetic control of human immune responses, the inheritance of several genes known to play important roles in a variety of immune processes has been analyzed in human families. HLA and T cell receptor (TCR) genes have been examined by Southern blot analyses using specific DNA probes to identify restriction fragment length polymorphism (RFLP). These studies have shown that there is limited polymorphism of TCR genes detected by RFLP in that few restriction enzymes reveal polymorphism with a given probe and when polymorphism is observed there are few allelic forms of each marker. However, there is considerable polymorphism in the combination of markers inherited together in both TCR α and TCR β haplotypes. These observations have an important impact upon studies to determine whether TCR genes are associated with disease susceptibility. TCR and disease association studies will most informatively be performed in families showing a predisposition to a particular disease. There is compelling evidence that susceptibility to Multiple Sclerosis is inherited and that T lymphocytes play a role in the pathogenesis of the disease. HLA and TCR haplotypes were determined in sibships with two or more members concordant for MS. Of the 16 families studied to date, 4 affected sib-pairs shared both TCR β haplotypes, 11 pairs shared 1 haplotype and 1 pair shared 0 haplotypes. In addition to the preponderance of shared haplotypes by patients there was also a TCR β RFLP which was present at an increased frequency in unrelated MS patients. Correlation between RFLP and sequence difference in TCR β V region coding sequences was examined by amplification of genomic DNA using the polymerase chain reaction (PCR), cloning into m13, and DNA sequence analysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI-00525-01-LIG
PERIOD COVERED October 1, 1987 to September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Analysis of Human Killer T Cells		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Eric O. Long Roberto Biassoni Rafick Sekaly	Visiting Scientist Visiting Fellow Visiting Fellow LIG, NIAID LIG, NIAID LIG, NIAID
COOPERATING UNITS (if any) National Institute for Cancer Research, Genoa, Italy (S. Ferrini, A. Moretta, L. Moretta)		
LAB/BRANCH Laboratory of Immunogenetics		
SECTION Molecular Immunology		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 2.5	PROFESSIONAL: 1.5	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Peripheral blood lymphocytes grown in the presence of high doses of interleukin-2 (IL-2) acquire the ability to lyse tumor cells. This cytolytic activity is not restricted by antigens encoded in the major histocompatibility complex (MHC) and is observed with a variety of both autologous and allogeneic tumors. This activity has been called lymphokine-activated killing (LAK) and has been used in clinical treatment of tumors. The origin of cells with LAK activity and the mechanism by which they recognize and kill their targets are unknown. More than 20 clones isolated from human peripheral blood were analyzed for their ability to lyse fresh tumor cells and for their cell-surface phenotype. Most of the clones with LAK activity had the CD3⁺CD4⁺CD8⁺CD16⁺ phenotype. Populations of cells with this phenotype were purified by a combination of antibody-mediated complement killing and cell sorting with the fluorescent-activated cell sorter. RNA extracted from clones and enriched populations of cells with LAK activity was analyzed by hybridization with DNA probes for the presence of transcripts derived from the CD3 and TCR genes. Absence of mature transcripts from the CD3γ, CD3δ, TCRβ, and TCRγ genes showed that recognition of tumor cells was not mediated by the known CD3-TCR α-β or γ-δ complexes. Interestingly, all CD3⁺ cells with LAK activity expressed the CD3ϵ and TCRδ genes. This finding identified these cells as members of an early stage in the T-cell lineage. Expression of genes encoding various lymphokines in activated CD3⁺CD4⁺CD8⁺CD16⁺ cells was measured. The pattern of lymphokine expression was also consistent with an early stage of T-cell maturation. These studies identified for the first time that cells with LAK activity belong in the T-cell lineage; and furthermore, that they represent the earliest known stage in T-cell differentiation.		

LABORATORY OF IMMUNOLOGY
1988 Annual Report
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PHS-NIH
Summary Statement
Office of the Chief
Laboratory of Immunology
October 1, 1987 through September 30, 1988

Introduction

The Laboratory of Immunology is concerned with the elucidation of the fundamental mechanisms underlying immunologic responses. It has made rapid progress through the use of new technologies that have created a revolution in immunologic science. These are the techniques of modern molecular biology, monoclonal antibodies, long term lines of cloned normal and transformed lymphocytes, and flow cytometric analysis. These methods combined with newer techniques in protein chemistry and cell biology should allow the solution of many of the major problems that have concerned immunologists and should lead to important advances in efforts to regulate the normal and the disordered immune response.

Evolutionary Conservation of Structure of Sterile C μ Transcripts

Sterile transcription of immunoglobulin genes may signify an "open" state of chromatin and has been suggested to represent a prelude to recombination events involving these genes. Such sterile transcription is known to occur for VH genes and for several CH genes. Laboratory of Immunology scientists have examined cDNA clones representing sterile C μ transcripts obtained from a rabbit splenic cDNA library. They demonstrated that this cDNA originates from a position 80 bp 3' of the conserved octamer sequence in the JH-C μ enhancer region. From the cDNA sequence, it was inferred that the mRNA was spliced from a donor site 635 bp 3' of the octanucleotide sequence to the C μ splice acceptor site. Analysis of human and murine Ig regions indicates preservation of the splice donor site in the comparable intron region in these species; evidence exists that some human and murine cell lines express spliced sterile C μ transcripts. The striking evolutionary conservation of the spliced mRNA strongly suggests that the production of such sterile transcripts plays an important role, beyond merely indicating the open state of chromatin, in the development of mature Ig genes or in switch recombination in B cells. (Mage, LI/NIAID)

Pairing of T Cell Receptor (TCR) α and β Chains Shows Striking Selectivity Suggesting A Limitation in TCR Repertoire

Laboratory of Immunology scientists have demonstrated that the specificity of T cells for antigen is fully defined by the TCR α and β chains they express. This was demonstrated by DNA-mediated gene transfer experiments in which genes specifying α and β chains were expressed in a T cell line which, as a result, acquired the specificity of the original donor of the transferred genes. A similar situation presumably holds for γ and δ chains in γ/δ expressing T cells. In the course of such studies, it was observed that when α and β chain genes were transfected into cells that already possessed α and/or β chain genes, the pattern of surface expression of α/β heterodimers was not random. This was monitored by the use of monoclonal antibodies specific for a given chain and the level of surface expression was compared to the level expression of mRNA encoding that chain. In extreme cases, it required 20-fold more mRNA (and presumably intracellular protein) to obtain equivalent cell surface expression in a TCR heterodimer. Most of the variability in the pairing of α and β chains appears to be due to differences in the structure of germline V β segments rather than to D or J segments or to the N region diversity created in the joining of gene segments. Comparable experiments were also carried out to determine whether mixed pairs occurred in cells that expressed genes for α , β , γ

and δ chains. In these cases, isotypic chain preference in pairing was virtually complete; no interisotypic pairing was observed. These results indicate that there is a requirement for a degree of structural complementarity in the pairing of the TCR chains and that in the α, β -expressing TCRs, the structure of the $V\beta$ chain plays a key role. These experiments thus imply that the repertoire of TCR molecules is more limited than would be postulated by simple combinatorial association and that there must have been some evolutionary pressure to accept preference in pairing. It seems likely that such pressure may have been the need to preserve certain critical receptors that were capable of recognizing some agent of particular importance to the species. Continued exploration of the linkage between T cell receptor structure and both specificity and expression promises to yield important information about the nature of the interaction between TCR and the MHC/antigenic peptide ligand and thus to allow more precise efforts at enhancement or elimination of responses of given specificities. (Saito and Germain, LI/NIAID).

Identification of a Region of the Class I Major Histocompatibility Complex K^b That is a Major T Cell Receptor Epitope

Class I major histocompatibility complex (MHC) molecules are co-recognized with antigenic peptides by T cell receptors of CD8+ T cells. This process appears to be analogous to the formation of the ternary complex between antigenic peptide, class II MHC molecule and TCR that is a feature of antigen recognition by CD4+ T cells. Laboratory of Immunology scientists have directly determined the critical site on the K^b molecule that is recognized by the majority (perhaps all) alloreactive cytotoxic T lymphocytes that lyse K^b -bearing target cells.

These studies began with the preparation of soluble forms of class I MHC molecules by the creation of chimeric genes consisting of the exons that specify the extracellular domains of conventional class I molecules and exons that specify the carboxy-terminal portion of a normally secreted class I molecule (Q10). Large amounts of various soluble class I molecules were prepared and tested for their ability to stimulate or inhibit alloreactive T cells. A multivalent form of the $D^d/Q10$ molecule proved capable of stimulating IL-2 production by a D^d -specific T cell hybridoma. Soluble $K^b/Q10$ was directly shown to interact with a K^b -specific T cell hybridoma. This was based on the capacity of the $K^b/Q10$ molecule to inhibit the response of the hybridoma to cells bearing mutant K^b molecules. Such mutant cells stimulated the hybridoma less vigorously than did the wild type, making inhibition more feasible. The concentration of soluble $K^b/Q10$ required for inhibition was $0.1 \mu M$, suggesting an equilibrium constant of the interaction of $\sim 10^7 M^{-1}$. This represents the first measurement of this binding event by a direct, quantifiable technique. Since the K^b mutants stimulated less vigorous responses than the wild type K^b cells, it was assumed that the site of the mutation would impinge on a (the) site recognized by the TCR. Thus, a peptide corresponding to the sequence of the K^b molecule in this region (residues 163-174) was synthesized and shown to also inhibit the stimulation of the T cell hybridoma by mutant cells, although much higher concentrations were required. Even more dramatically, it was shown that primary allogenic cytotoxic T cell responses specific for K^b were specifically inhibited by the peptide indicating that the site represented by residues 163-174 was the region recognized by virtually all K^b allospecific cytotoxic T cells. This work is of great importance in understanding the structures recognized by T cell receptors and will be critical to further progress in rational vaccine design. (Schneck, McCluskey, Inman and Margulies, LI/NIAID; Coligan, BRB/NIAID; Munitz and Singer, IB/NCI).

Structure-Function Analysis of Class II Major Histocompatibility Complex (MHC) Molecules

Class II MHC molecules are heterodimers consisting of α and β chains that act as key elements in the process by which CD4+ T lymphocytes recognize and respond to antigens. These MHC molecules bind peptides derived from foreign antigens and the complex of class II molecule

and antigenic peptide is recognized by the T cell receptor (TCR). Laboratory of Immunology scientists have been attempting to elucidate the relation of the structure of class II molecules to three functional characteristics of these molecules: 1) the pairing of α and β chains which shows both "allotypic" and "isotypic" preferences; 2) the binding of antigenic peptides to the class II molecule; and 3) the binding of the TCR to the class II molecule/antigenic peptide complex. A related and important issue is the binding of CD4 molecules to class II MHC molecules and the potential competition between class II MHC molecules and the envelope protein of the human immunodeficiency virus in binding to CD4.

It has been demonstrated that α and β chains encoded by the same class II locus (I-A or I-E, respectively) pair preferentially to one another. This isotypic pairing preference accounts for the failure to observe molecules consisting of chains encoded in I-A pairing with β chains encoded in I-E. Similarly, α chains tend to pair preferentially with β chains specified by cis encoded genes in the same region (allotypic pairing). Quantitative studies of the structural basis of these pairing preferences using DNA-mediated gene transfer of chimeric genes and of mutant genes, obtained by site-directed mutagenesis, have indicated that the amino-terminal portion of both the α and β chains play key roles in pairing preference and, based on inferences from the crystal structure of a human class II molecule, the "preference determining" residues are located on interior strands of the assembled molecule. Other efforts have been directed at identifying regions of the class II molecules which participate in the binding of antigen peptides. Based on testing functional properties of transfectants that receive either wild type or site-directed mutant E β genes, residues 29, 72 and 75 of the β chain appeared to play an important role in determining the specificity of class II-antigen interaction. Interestingly, studies based on the capacity of T cells to recognize the class II molecule together with an antigenic peptide suggest that residue 79 of the β chain may play an important role in this process. This work promises to yield very important information of the chemical basis of the formation of the ternary complex of antigen-MHC molecule with TCR that is central to the activation of T cells and may have very important implications in the design of vaccines that can lead to T cell activation. (Sant, Ronchese and Germain; LI/NIAID)

Multiply Conjugated High Molecular Weight Soluble Polymers as Tools to Study Cellular Activation Events Requiring Receptor Cross-Linkage

The activation of specific lymphocytes by antigen depends upon cross-linkage of cell membrane receptor molecules. Cross-linkage can be achieved by bivalent molecules, such as anti-receptor antibodies or by oligovalent antigens, such as lightly substituted hapten-protein conjugates. Activation resulting from such cross-linkage tends to be of relatively small magnitude and requires large amounts of ligand, far beyond what appear to be physiologically meaningful concentrations. On the other hand, very efficient activation of both B and T lymphocytes can be achieved by expressing anti-receptor antibodies or other ligands on solid matrices. Unfortunately, it is very difficult to measure effective concentrations of ligands on such surfaces and estimation of spacing of ligands are also quite uncertain. LI scientists have prepared high molecular weight dextrans and Ficolls and high molecular weight linear polyacrylamide multiply substituted with anti-receptor antibodies or with soluble class II major histocompatibility complex molecules. These soluble, highly multivalent ligands are very efficient stimulants of cellular activation. For example, high molecular weight soluble dextran bearing ~20 anti-IgD antibodies per 2×10^6 daltons of polymer stimulates B cell DNA synthesis at a concentration of anti-IgD 1000-fold lower than required when soluble anti-IgD itself is used. These concentrations are in the range of the concentrations of type 2 antigens (e.g. hapten-Ficoll conjugates) required for antibody responses suggesting that they reflect physiologic stimulatory conditions. This approach has also been used for stimulation of an alloreactive T cell hybridoma. A soluble form of the D^d class I molecule substituted on to high molecular weight dextran stimulated production of interleukin-2 by these cells, indicating that such

cross-linkage was sufficient for cellular signal transmission and activation. This approach should have broad usefulness in analysis of immune recognition and, indeed, in all biological recognition systems in which receptor cross-linkage plays a major role. In particular, it may allow a direct test of the concept that ordered cross-linkage may have particular importance, such as through the formation of particular cell surface structures that mediate signal transduction. (Inman, Schneck and Margulies, LI/NIAID; Brunswick, Finkelman and Mond, USUHS; Dintzis, Johns Hopkins School of Medicine).

T Lymphocytes and B Lymphocytes Express Strikingly Different Regulation of Intracellular Free Calcium Concentration

Resting intracellular free calcium concentration $[Ca^{++}]_i$ is ~100 nM while extracellular Ca^{++} concentrations are ~1 mM. Laboratory of Immunology scientists have shown that T and B lymphocytes show strikingly different management of $[Ca^{++}]_i$ under conditions of Ca^{++} influx, which are often critical elements of cellular activation. To examine this, they utilized calcium ionophores and demonstrated that ionomycin at concentrations between .25 and .8 μ M causes a stable elevation of $[Ca^{++}]_i$ in B cells, in the range of 150 to 500 nM. By contrast, T cells exposed to the same concentrations of ionomycin achieve $[Ca^{++}]_i$ that are 5 to 10-fold higher. This striking difference in Ca^{++} management depends, in part, on a much more active Ca^{++} pump in B cells than in T cells, as demonstrated by measurements of ^{45}Ca efflux. Furthermore, T cells possess a Ca^{++} -sensitive K^+ channel; at $[Ca^{++}]_i$ levels above 300 nM, increased K^+ permeability results in hyperpolarization of T cells whereas B cells depolarize when $[Ca^{++}]_i$ is elevated. If induced hyperpolarization is prevented by increasing extracellular K^+ concentration prior to the addition of ionomycin, T cell $[Ca^{++}]_i$ is greatly diminished and the difference in $[Ca^{++}]_i$ levels achieved in T and B cells in response to ionomycin is markedly decreased. These results suggest that membrane potential regulates that action of the Ca^{++} pump. The striking differences in Ca^{++} management in B and T cells may indicate a distinctiveness in their responsiveness to receptor cross-linkage and may allow independent manipulation of the responses of these two cell types. (Ishida and Chused, LI/NIAID)

Exocytosis of Granules of Cytotoxic Lymphocytes if Not Required for Killing Event

Cytotoxic T lymphocytes (CTL) express secretory granules that contain a variety of enzymes and other proteins that have been considered important candidates for mediation of the cytotoxic function of CTL. The release of granules from CTL is triggered by receptor cross-linkage and appears to involve the activation of protein kinase C, the elevation of cytosolic $[Ca^{++}]$, the translocation of extracellular Ca^{++} into the cell, and the function of calmodulin and calmodulin-binding proteins. In an effort to understand this process more completely, Laboratory of Immunology scientists have examined the calmodulin-binding proteins of lymphocytes. They have shown that the principal calmodulin-binding protein is a calmodulin-dependent phosphatase, calcineurin. This suggests that dephosphorylation may be a principal outcome of elevation of cytosolic $[Ca^{++}]$ in CTL.

It was demonstrated that the exocytosis of CTL granules that results from either receptor cross-linkage or from stimulation of CTL with pharmacologic agents that elevate calcium (calcium ionophores) and activate protein kinase C (phorbol esters) have an absolute requirement for extracellular Ca^{++} . Nonetheless, as LI scientists showed, CTL-mediated target cell lysis could be obtained in the absence of extracellular Ca^{++} , establishing that, despite the attractiveness of this theory of cell-mediated cytotoxicity, granule release was not required for lysis. Furthermore, the release of newly synthesized interferon gamma from CTL also required extracellular Ca^{++} , suggesting that such secretion was also not essential for the lytic action of CTL. This work indicates that cytotoxic mechanisms not requiring translocation of calcium into

the CTL play a important role in the function of CTL. Efforts to delineate such mechanisms are now in progress. (Tren, Takayama and Sitkovsky, *LI/NIAID*)

Mast Cell and Myeloid Cell Lines Produce Lymphokines When Stimulated with Ionophores or When Fc Receptors are Cross-Linked

T cell derived lymphokines including interleukin-3 (IL-3), interleukin-4 (IL-4) and interleukin-5 (IL-5) have profound effects on many cells on the immune system and other hematopoietic lineage cells. Laboratory of Immunology scientists have now demonstrated that long term, but non-transformed, lines of both mast cell and myeloid lineage can also produce these lymphokines. Previous work in the Laboratory of Immunology had shown that a majority of transformed mast cell lines expressed mRNA for IL-4 and that these lines secreted IL-4. The growth of these transformed cells was not dependent upon autocrine production of IL-4 and addition of IL-4, which is a known co-stimulant of the growth of factor-dependent mast cells, had no effect on the growth of cells of the transformed lines. For this reason, LI scientists considered the possibility that IL-4 expression in transformed mast cells represented the range of products produced by the non-transformed mast cells. LeGros, LeGros and Watson had reported that a myeloid line, FDC.1, secreted IL-3, IL-4 and GM-CSF when stimulated with immune complexes. That led to the consideration that a common mechanism might control lymphokine production in non-lymphocytic hematopoietic cells. The observation that immune complexes stimulated lymphokine production by FDC.1 cells was confirmed and extended. It was shown that this effect was due to the cross-linkage of the Fc γ RII receptor on the myeloid cell since a soluble monoclonal antibody (2.4G2) to this receptor inhibited lymphokine production and 2.4G2 immobilized on a surface stimulated release. The production of IL-3 and IL-4 by FDC.1 cells was inhibited by Cyclosporin A and stimulated by calcium ionophores, suggesting that it required new transcription of lymphokine genes and that elevation of cytosolic [Ca⁺⁺] was a sufficient signal for this event. This work was then extended to factor dependent mast cell lines derived from fetal liver. CFTL.12 cells developed striking upregulation of IL-3, IL-4 and IL-5 mRNA levels when stimulated with ionomycin; little IL-2 mRNA was noted. Furthermore, sensitization of the Fc ϵ receptors of these cells with monoclonal IgE anti-DNP antibodies caused secretion of IL-3 in response to a multivalent DNP-protein, which cross-linked the membrane Fc ϵ receptors. These cells also showed substantial increases in mRNA for IL-3 and IL-5 and a doubling of IL-4 mRNA.

These results indicate that mast cells and myeloid cell lines can produce substantial amounts of several, although perhaps not all, "T cell-derived" lymphokines. Furthermore, physiologic events involving cross-linkage of Fc receptors are potent stimuli for lymphokine production. The capacity of mast cells to secrete lymphokines that regulate mast cell growth (IL-3 and IL-4), switching to the expression of IgE (IL-4), and differentiation of eosinophils (IL-5) suggests that mast cells may participate in allergic inflammatory responses in a much more central manner than simply by releasing vasoactive amines. This may suggest strategies for controlling allergic responses and for enhancing protective immunity to parasites. (Plaut, LeGros and Paul, *LI/NIAID*; Pierce, *LCMB/NCI*)

Dendritic Epidermal T Cells Display T Cell Receptors of the $\gamma\delta$ Type and Adhere to Matrices Through Members of the Integrin Superfamily of Cell Adhesion Molecules

Dendritic epidermal T cells (DETC) are a population of Thy-1+ epidermal bone marrow-derived cells that generally fail to express T cell receptors (TCR) consisting of α/β heterodimers. Laboratory of Immunology scientists have demonstrated that this interesting and potentially very important cell population does express TCR that utilize $\gamma\delta$ heterodimers. Careful characterization of the usage of these chains indicates that considerable variability among distinct DETC exists in the precise γ genes used and in the structure of the δ chain,

indicating that this cell type can express considerable diversity in its repertoire of expressed receptors. Furthermore, two of the DETC lines expressed two distinct TCR γ chains, derived from distinct γ loci, suggesting that isotypic exclusion does not always occur for γ genes and proteins.

Among a set of monoclonal antibodies to DETC, two were identified that immunoprecipitate a molecule that appears to be a member of the integrin superfamily of cell adhesion molecules (I-SCAM). The binding of DETC to fibronectin coated plates is inhibited by the tetrapeptides consisting of Arg-Gly-Asp-Ser and is also blocked by both monoclonal antibodies. The monoclonal antibodies also blocked the binding of DETC to surfaces coated with vitronectin, collagen or fibrinogen. These specificity characteristics suggest that the surface molecule is similar to the human VLA-3 antigen. Although normal mouse lymphocytes fail to bind these monoclonal antibodies when freshly derived from an animal, several long term lines of T cells do react with the antibodies and their reactivity is enhanced by antigen-specific activation. These reagents should be of great value in understanding lymphocyte adhesion properties and in clarifying lymphocyte homing and development in vivo. (Yokoyama and Shevach, LI/NIAID)

IL-4 Controls the Production of IgE Both In Vitro and In Vivo

IL-4 is a T cell-derived lymphokine, also known to be produced by some mast and myeloid cell lines. Laboratory of Immunology scientists discovered this lymphokine in experiments aimed at understanding the requirements for stimulation of resting B cells to proliferate when their membrane Ig was cross-linked. Since that initial description, IL-4 has been shown to have a wide range of biologic activities. It acts on both B and T lymphocytes, as well as on cells of virtually every hematopoietic lineage and on some non-hematopoietic cells. IL-4 has been purified to homogeneity, cDNA clones for IL-4 have been obtained, and IL-4 has been expressed in a variety of expression systems. This year LI scientists have obtained large amounts of IL-4 by preparing a recombinant baculovirus in which the IL-4 gene has been substituted for the polyhedrin gene. This has made possible the production and purification of milligram quantities of IL-4; in addition, a monoclonal anti-IL-4 antibody has been derived and large amounts of the antibody have been produced, sufficient for in vivo experiments to test physiologic functions of IL-4.

Treatment of resting B cells with IL-4 and lipopolysaccharides causes a series of striking effects on the B cells. These include upregulation of expression of class II major histocompatibility class molecules and of Fc ϵ RII molecules as well as induction of Thy-1 on B cells and inhibition of expression of Fc γ RII. In association with this is the appearance of membrane IgG1 on ~50% of the B cells and of membrane IgE on 15 to 25% of the cells. Indeed, 2/3 of the cells that express mIgE also express mIgG1 and these cells expressing two surface isotypes can be purified by fluorescence-activated cell sorting. The resultant cells give rise to IgG1 and to IgE producing cells when returned to culture with LPS and additional IL-4. This indicates that "double positive" cells retain the genetic information for the secretion of IgG1 and suggests that in their expression of mIgE, they have not deleted a functional IgG1 gene. Analysis of amounts of IgE produced indicates that IL-4 costimulation with LPS can cause secretion of very large quantities of IgE (up to 5 μ g/ml).

IL-4 action can be inhibited in vivo by the administrations of large amounts of monoclonal anti-IL-4 antibodies. The efficiency of this approach is demonstrated by the observation that elevation of expression of class II MHC molecules in certain in vivo models is blocked by anti-IL-4 antibody. Monoclonal anti-IL-4 antibody profoundly inhibits (~99%) both primary polyclonal IgE responses that ensue in *Nippostrongylus*-infected mice and in mice injected with anti-IgD antibodies; it also inhibits specific secondary IgE responses in animals primed with alum. It has no effect on polyclonal primary or secondary responses of other isotypes, including IgG1, upon which it has profound in vitro effects. These results suggest that IL-4 may be an obligatory factor in IgE production and, possibly, that in vivo its unique range of actions may

be sufficiently limited so that interference with the IL-4 pathway of stimulation may be useful for the control of allergic and parasitic disorders. (Snapper, Ohara and Paul, LI/NIAID; Katona and Finkelman, USUHS; and Urban, Department of Agriculture.

In Vivo T Cell Responses to Allogeneic Cells are not Inhibited by Cyclosporin A Suggesting the Use of Novel T Cell Proliferation Pathways

Cyclosporin A is a potent immunosuppressive drug which mediates many of its functions through blocking the stimulation of lymphokine transcription. Indeed, the activation of lymphocytes in draining lymph nodes that is observed as result of injection of anti-CD3 antibody into the footpad is largely inhibited by administration of cyclosporin A. By contrast, if allogeneic cells are injected in footpads, there is vigorous cellular enlargement and expression of IL-2 receptors in cells within the draining lymph node and these cells show substantial "spontaneous" proliferation and IL-2 production when freshly explanted. Administration of cyclosporin A to such animals does not inhibit these responses although it does appear to block in vivo IL-2 production since these cells do not show any stimulation of IL-2 mRNA expression. When the cells are explanted in the absence of cyclosporin A, they produce IL-2; this IL-2 production is inhibited if cyclosporin A is added to the culture. Although these cells fail to secrete IL-2, their proliferation is not inhibited. Monoclonal antibodies to a series of other lymphokines also fails to block proliferation of T cells that had been primed in vivo to allogeneic cells in the presence of cyclosporin A. These studies indicate a mechanism of T cell activation that is not inhibited by cyclosporin A which may be mediated by an as yet unidentified lymphokine or possibly by the effect of antigen itself. In addition, this work strikingly points out that spontaneous IL-2 production begins when cyclosporin A is removed indicating the potential danger of terminating cyclosporin A treatment. (KroczeK and Shevach, LI/NIAID).

Honors, Awards and Scientific Recognition

Laboratory of Immunology scientists play important roles in the U.S. and international immunological communities. Dr. William E. Paul served as president of the American Association of Immunologists and was the recipient of the 3M Life Sciences Award from the Federation of American Societies for Experimental Biology. Dr. Ethan Shevach is the editor-in-chief of the *Journal of Immunology* and was chairman of the Gordon Research Conference on Immunochimistry and Immunobiology. Dr. Ronald Germain was organizer and chairman of the Sixth HLA/H2 Cloning Workshop.

Laboratory Scientists serve on editorial boards of many scholarly publications. In addition to his role as editor-in-chief of the *Journal of Immunology*, Dr. Shevach is a member of the editorial boards of *Cellular Immunology* and of the *Journal of Immunological Methods*. Dr. Paul is the editor of the *Annual Review of Immunology* and is an advisory editor of the *Journal of Experimental Medicine*, an associate editor of *Cell*, and a member of the editorial boards of *Immunological Reviews* and of the *Journal of Molecular and Cellular Immunology*. He is consulting editor for immunology of the *Cecil Textbook of Medicine*, a member of the advisory editorial board of the *ISI Atlas of Science* and a transmitting editor of *International Immunology*. Dr. Germain is a deputy editor of the *Journal of Immunology* and is a member of the advisory editorial board of the *Journal of Molecular and Cellular Immunology* and of the *Annales de l'Institut Pasteur*. He served as an acting associate editor of the *Annual Review of Immunology*. Dr. Mage is a member of the editorial board of *Immunogenetics*. Dr. Inman is a member of the editorial board of *Analytical Biochemistry*, is an advisory editor of *Molecular Immunology* and is a member of the newsletter staff of *ISBT Communications*. Dr. Chused is a member of the editorial board of *Cytometry*.

Dr. Paul is a member of the Board on Basic Biology of the National Research Council and this board's Committee on Research Opportunities in Biology. He completed a term as a member of the board of directors of the Federation of American Societies for Experimental Biology. Dr. Paul is a member of the committee to visit the Division of Medical Sciences of the Board of Overseers of Harvard College and of the advisory committee of the Pew Scholars Program in the Medical Sciences and of the selection committee for the RJR/Nabisco Scholars Award in Immunology. He is a member of the scientific review board of the Howard Hughes Medical Institute, of the board of scientific advisors of the Jane Coffin Childs Fund for Medical Research, of the board of scientific consultants of the Memorial-Sloan Kettering Cancer Center, of the scientific advisory committee of the Cancer Research Institute, Inc., and of the board of directors of the Foundation for Advanced Education in the Sciences. He is chairman of the advisory committee of the Harold C. Simmons Arthritis Research Center.

During the past year, Dr. Paul chaired and presented a lecture at the Plenary Symposium "Frontiers of Biomedical Research" at the Centennial of the National Institutes of Health. He gave the 3M Lecture at the annual meeting of the Federation of American Societies for Experimental Biology in Las Vegas. He chaired a symposium and presented an invited lecture at the Congress on Cytokine Research, in Philadelphia, PA. He chaired the Ciba-Geigy Drew symposium on New Pharmacological and Physiological Approaches to Immunoregulation and was an invited lecturer at the New York Academy of Sciences conference on the Molecular Basis of the Immune Response; at the UCLA Conference on B Cell Development in Taos, NM; at the UCLA Conference on Molecular and Cellular Mechanisms of Human Hypersensitivity and Autoimmunity at Keystone, CO; at the symposium at College of Physicians and Surgeons of Columbia University honoring the centennial of Michael Heidelberger's birth; and at the Symposium on Chemically Induced Autoimmunity of the Immunotoxicology Discussion Group.

In addition to his role as chairman of the Gordon Research Conference on Immunochemistry and Immunobiology, Dr. Shevach was an invited lecturer at the annual meeting of the American Society of Microbiology; at the Midwinter Conference of Immunologists, Asilomar, CA; at the Klenk Conference on Immunology/Neurobiology; and at the Conference on Immunity to Cancer.

Dr. Germain serves as a member of the Advisory Committee on Clinical Investigations I-Immunology and Immunotherapy of the American Cancer Society and as a member of the Clinical Review Subpanel of the National Institute of Allergy and Infectious Diseases. Dr. Germain was a plenary speaker at the 10th Histocompatibility Workshop of the American Society for Histocompatibility Investigation; an invited lecturer at the meeting "Molecular Basis of Antigen Presentation by MHC Molecules" in Paris, France, sponsored by the Pasteur Institute; keynote speaker and special consultant at the World Health Organization working session on "Exoerythrocytic and Asexual Blood Stage Vaccines" in Geneva, Switzerland; session chairman at the Gordon Research Conference on Immunochemistry and Immunobiology; special lecturer at the Marine Biological Laboratory course on Biology of Parasitism; and an invited speaker at the meeting on Pattern Recognition by T Cells sponsored by the Cancer Research Institute, Inc.

Dr. Mage serves as the American Association of Immunologists representative to the board of the American Type Culture Collection. She chaired a symposium on Evolution and Function of Antigen Recognition Mechanisms at the International Congress of Biochemistry in Prague, Czechoslovakia and was an invited speaker at the symposium on Immunoglobulin Gene Repertoire and Regulation at the Midwest Autumn Immunology Conference.

Dr. Margulies is a member of the Immunology and Immunotherapy Advisory Committee of the American Cancer Society.

Dr. Sitkovsky was a plenary speaker at the International Workshop on Cellular Cytotoxicity and made a presentation at the International Workshop on "Calcium in Health and Disease" in Nagoya, Japan.

In addition, Laboratory of Immunology scientists presented seminars at major universities, medical schools and research institutes in the United States and abroad.

Administrative, Organizational and Other Changes

The Laboratory of Immunology adopted a sectional structure during the past year. The sections established were: Cellular Immunology Section, Ethan M. Shevach, Section Head; Lymphocyte Biology Section, Ronald Germain, Section Head; Molecular Immunogenetics Section, Rose G. Mage, Section Head; Molecular Biology Section, David H. Margulies, Section Head; Transmembrane Signalling Section, Thomas Chused, Section Head; Bioorganic Chemistry Section, John K. Inman, Section Head. This sectional structure should allow a more efficient operation of the Laboratory and provides appropriate recognition for scientists who have made a major contributions to the Institute's Intramural Research Program. During the past year, Dr. Richard Asofsky joined the Laboratory of Immunology as a senior investigator and head of the Experimental Pathology Section and Dr. David Scott joined the Laboratory as a senior investigator.

The Laboratory of Immunology is a major training center for young immunologists. This was made clear when many of the former members and post-doctoral fellows of the Laboratory returned for the NIH Centennial celebration. Three of the Laboratory's "alumni", Baruj Benacerraf (Harvard Medical School), Mark Davis (Stanford University School of Medicine) and John Stobo (Johns Hopkins University School of Medicine) presented invited lectures at the Immunology Symposium held as part of the Centennial.

During the past year several scientists completed post-doctoral training or sabbatical periods in the Laboratory of Immunology. Among them were Anne Fortier, Zvi Grossman, Nagaradona Harindranath, Richard Lopez, Kumeo Ono, Christian Peschell, Marshall Plaut, Kathleen Melez, Takashi Saito, Clifford Snapper, Hajime Takayama, Guido Trenn, and Wayne Tsang. In addition, Mark Jenkins and Drew Pardoll who began post-doctoral training in the Laboratory of Immunology completed that training this year in the newly established Laboratory of Cellular and Molecular Immunology to which they had transferred with their mentor Dr. Ronald Schwartz. Each of these scientists made important contributions to the Laboratory research program.

During the past year, several scientists joined the Laboratory of Immunology for post-doctoral training or sabbatical visits. They included Takashi Agui, Masimo Allegrucci, John Classen, Dan Conrad, Antonio Filippini, Graham LeGros, Steven Maxfield, Masako Okajima, Jeffrey Sherman and Hirota Sugiyama. It is anticipated that they will continue a tradition established by a long series of outstanding trainees in the Laboratory.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00030-20 LI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antigen Recognition and Activation of Immunocompetent Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	William E. Paul	Chief	LI/NIAID
Others:	Wayne Tsang	Medical Staff Fellow	LI/NIAID
	Peter Hornbeck	Staff Fellow	LI/NIAID
	Graham LeGros	Visiting Fellow	LI/NIAID
	David Margulies	Senior Investigator	LI/NIAID
	Thomas Chused	Senior Investigator	LI/NIAID
	S.Z. Ben Sasson	Visiting Scientist	LI/NIAID

COOPERATING UNITS (if any)

National Institute of Child Health and Human Development (K. P. Huang;
National Institute of Mental Health (D. Kligman).

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

4.4

PROFESSIONAL:

3.4

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Membrane immunoglobulin (mIg) acts as the surface receptor used by B lymphocytes for recognition of antigens. Cross-linkage of mIg leads to activation of the inositol phospholipid metabolic pathway, elevation of cytosolic Ca^{2+} concentration, and protein kinase C-mediated protein phosphorylation. These events can, in part, be mimicked by the use of ionomycin and phorbol myristate acetate (PMA), pharmacologic agents that elevate cytosolic Ca^{2+} and that activate protein kinase C. We have recently undertaken a study of the protein substrates phosphorylated in response to PMA and to agents that cross-link mIg. Two of these appear to be of special interest. One, lamin B, is a nuclear filament protein that is the major nuclear species phosphorylated in cells treated with PMA. Lamin B is phosphorylated in vivo in a pattern very similar to that observed for in vitro phosphorylation of partially purified lamin B by PKC, suggesting that PKC or a PKC-like kinase gains access to the nucleus and phosphorylates substrates at that site. This provides a means for the rapid transmission of surface signal to the nucleus. The second major substrate pp65-70 is one of the major myristylated proteins of the cell. pp65-70 is expressed in substantial amounts in B cells and B lymphomas such as WEHI-231 but mature T cells fail to express pp65-70. Immature thymocytes display large amounts of pp65-70 while more mature thymocytes express only small amounts of pp65-70 indicating that this PKC-substrate is developmentally regulated.

The means through which receptor cross-linkage leads to activation of cellular biochemical signals is still unresolved. Efforts to understand the molecular requirements for signal generation through the creation of chimeric genes that express the external domains of class I or class II MHC molecules and the transmembrane and cytoplasmic domains of Ig (μ , δ , $\gamma 2b$) and the examination of their capacity to transmit signals are in progress.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00035-13 LI

PERIOD COVERED

October 1, 1987 to September 31, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Specificity in Immune Responses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. K. Inman
Others: P. F. HighetSection Head
TechnicianLI, NIAID
LI, NIAID

COOPERATING UNITS (if any)

Dept of Medicine, USUHS (J. Mond, F. Finkelman, M. Brunswick);
Dept of Biophysics, Johns Hopkins University School of Medicine,
Baltimore, MD (H. M. Dintzis).

LAB/BRANCH

Laboratory of Immunology

SECTION

Bioorganic Chemistry Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.8

PROFESSIONAL

1.0

OTHER:

0.8

CHECK APPROPRIATE BOXES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A major effort of this project is directed toward the design and synthesis of highly specific and efficient immunomodulators. The current design principle is based on the signal amplification produced by polyvalent presentation of effector molecules and is closely allied with the immunon construct of Dintzis et al. Effector molecules (e.g., monoclonal antibodies directed against specific cell surface proteins) are multiply and covalently linked to soluble polymer carriers of very high molecular weight using heterobifunctional reagents. Various structural parameters are being varied in order to optimize the performance of these immunomodulators, and collaborative studies are in progress to explore their properties. So far these studies have dealt mainly with mitogenic stimulation of B cells by soluble conjugates of anti-IgD and anti-IgM. Conjugates of class I MHC antigens with high polymers have been employed in studies of T-cell receptor-MHC interactions. New methods are being explored for linking antibodies through their polysaccharide chains to high molecular weight carriers.

Another important aim of this project is to explore the property of general multispecificity of antibodies and other kinds of receptors. Studies are in progress to compare the frequency distribution of binding constants of monoclonal antibodies for large numbers of diversely structured substances. Additional studies will be directed at showing to what extent binding events from the above trials are representations of alternative modes of complementation in the binding site region of a given receptor. These findings will be related to specificity and other important functions of receptor-effector interactions and networks in the immune system.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00036-23 LI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ig Genetics: Ontogeny and Differentiation of Cells of the Rabbit Immune System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. G. Mage	Section Head	LI, NIAID
Others: B. Newman	IRTA Fellow	LI, NIAID
N. Hole	Visiting Fellow	LI, NIAID
N. Harindranath	Visiting Fellow	LI, NIAID
M. Allegrucci	Visiting Associate	LI, NIAID
R. Wilder	Senior Investigator	LI, NIAID

COOPERATING UNITS (if any): Basel Inst. Immunol., Basel, Switzerland (A. S. Kefau); Dept Microbiol. Univ. Illinois Med. Ctr., Chicago, IL (K. Knight); Dept Biochemistry, Univ. Geneva, Geneva, Switzerland (J. -C. Jaton); Nat'l Univ. Mexico, Mexico City (E. Lamoyi); Dept Biochem., Univ. Tokyo (A. Kobata).

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Immunogenetics

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.1

PROFESSIONAL:

2.1

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

We use classical immunogenetic techniques as well as techniques of molecular biology to study the genetics of rabbit immunoglobulins (Igs) and T cell receptors and the regulated expression of the genes that encode these molecules.

A rabbit cDNA copy of splenic mRNA is spliced to Cμ from a site 635 bp 3' of the conserved octanucleotide of the heavy chain enhancer. A splice site is present at a similar position in the intron sequences of mouse and man. There is evidence that such spliced sterile transcripts occur in developing B cells of mice and in some human cell lines. The evolutionary conservation of the spliced mRNA in three species suggests that it may play a functional role during B cell ontogeny.

We are characterizing T cell receptor Cβ genes from rabbits which have three copies of the Cβ constant region. We previously demonstrated that there are allotypes of rabbit Cβ1. We have now sequenced about 4 kb of a cloned 6 kb fragment from an animal of type b and demonstrated that this fragment contains a rabbit Cβ2 gene. Comparisons with the cDNA sequence from an animal of Cβ type a show replacement changes in the first and third exons thus demonstrating that there are also allotypic forms of Cβ2.

Decreased galactosylation of IgG that is observed in a sera of rheumatoid patients is also observed in the IgG prepared from sera of rabbits post-immunization with Streptococcal vaccines. Therefore the difference in IgG galactosylation seen in patients may also be induced rather than reflect an inherent genetic difference in individuals with rheumatoid disease.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00186-14 LI

PERIOD COVERED

October 1, 1987 to September 31, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis of Autoimmunity in Inbred Strains in Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. M. Chused
Others: E. M. BrownSenior Investigator
BiologistLI, NIAID
LI, NIAID

COOPERATING UNITS (if any)

Renu Lal, Visiting Fellow, LPD, NIAID; John Coligan, Chief, BRB, NIAID;
Philip J. Baker, Section Chief, LMI, NIAID; Herb Cooper, Section Chief, NCI.

LAB/BRANCH

Laboratory of Immunology

SECTION

Transmembrane Signalling Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00203-08 LI

PERIOD COVERED

October 1, 1987 to September 31, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Applications of Flow Cytometry

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. M. Chused Senior Investigator LI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

Transmembrane Signalling Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00224-07 LI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Monoclonal Antibodies as Probes for T Cell Activation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	E. M. Shevach	Section Head	LI, NIAID
Others:	W. Yokoyama	Senior Staff Fellow	LI, NIAID
	G. Chazen	Medical Staff Fellow	LI, NIAID
	G. Pereira	Visiting Associate	LI, NIAID
	K. Roberts	Visiting Associate	LI, NIAID
	K. Ono	Visiting Fellow	LI, NIAID
	D. Presky	Guest Worker	LI, NIAID
	S. Maxfield	Guest Worker/HMFI Fellow	LI, NIAID

COOPERATING UNITS (if any)

Dept of Dermatology, Univ Vienna, Vienna, Austria (G. Stingl); Biological Resources Branch, NIAID, NIH (J. Coligan); Laboratory of Chemical Biology, NIDDKD, NIH (D. Cohen)

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

8.0

PROFESSIONAL

6.0

OTHER

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The major objective of our studies is to characterize cell surface structures on T lymphocytes which, in addition to the antigen-specific receptor (TCR), play critical roles both in vivo and in vitro in the process of lymphocyte activation. The TCR as well as other cell surface antigens of Thy-1⁺ dendritic epidermal T cells (DETC), a unique population of cells comprising 1-2% of murine epidermis, have been extensively characterized. DETC bear exclusively $\gamma\delta$ -TCR; a combination of biochemical and molecular approaches has shown that both the TCR γ -chain and the TCR δ -chain expressed by this population of cells exhibit considerable diversity. A panel of monoclonal antibodies (mAbs) has been generated against DETC. One mAb recognizes a cell surface disulfide linked dimer of 85 kD under non-reducing conditions. In the presence of phorbol esters, this mAb is a potent stimulator of T cell activation. Its biochemical and functional properties suggest that this mAb identifies the murine homologue of the human CD28 antigen. A second group of mAbs identify members of the Integrin Superfamily of Cell Adhesion Molecules (I-SCAM). These mAbs specifically inhibit the binding of DETC to a number of extracellular matrix proteins.

We have also continued our studies of the Thy-1 and Ly-6 antigens which are coupled to the cell membrane via a phosphatidylinositol (PI) linkage. We have obtained evidence for additional PI-linked proteins which may play important roles in cell-cell interaction. An Ly-6-linked gene has been cloned and shown by transfection studies to be Ly-6A.2. Lastly, we have shown that T lymphocytes can be activated in vivo by allogeneic cells in the presence of the immunosuppressive drug, Cyclosporin A (Cy A) and that this activation process is independent of any of the known Interleukins.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00226-07 LI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Rabbit Allotypes: Structure, Organization and Regulated Expression of Ig Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	R. G. Mage	Section Head	LI, NIAID
Others:	B. Newman	IRTA Fellow	LI, NIAID
	N. Hole	Visiting Fellow	LI, NIAID
	N. Harindranath	Visiting Fellow	LI, NIAID
	M. Allegrucci	Visiting Associate	LI, NIAID

COOPERATING UNITS (if any) Basel Inst. Immunol., Basel, Switzerland (A. S. Kelus), Dept Microbiol., Univ. Illinois Med. Ctr., Chicago, IL (Dr. K. Knight); Dept Biochemistry, Univ. Geneva, Geneva, Switzerland (J. -C. Jaton); Nat'l Univ. Mexico, Mexico City (E. Lamoyi); Nat'l Inst. of Animal Industry, Ibaraki, Japan (M. Komatsu).

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Immunogenetics

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.2

PROFESSIONAL:

2.2

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We study genes of the rabbit immune system by techniques of molecular biology. The inheritance of serum allotypes of two rabbit C κ isotypes and restriction fragment polymorphisms (RFLPs) of the genes for V κ , C κ and the C β of the Tcr was studied through backcross and F2 matings. The rabbit resembles man in having unlinked C κ and C β loci whereas in mice these genes are linked at a distance of ~8 centimorgans.

We devised a simple and efficient method for determining RFLP types on large numbers of individuals using small samples of peripheral blood or sperm cells. The technique is readily adaptable to any application requiring DNA from small numbers of cells for Southern analyses including pulsed field gel electrophoretic studies. We are studying the low relative expression of the rabbit K2 light chain isotype. A physical map of the duplicated region containing two rabbit kappa light chain genes is being generated.

We are studying the atypical expression of VH α 2 genes in ali rabbits and the relationship of the mutated chromosome to a VH-CH recombinant chromosome. We are mapping the heavy chain regions of parental and recombinant chromosomes and have discovered a new VH-CH recombinant. The present studies place the site of F-I recombination upstream of JH.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of Murine Class II MHC Genes and Gene Products

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	R. Germain	Section Head	LI, NIAID
Others:	A. Sant	Special Volunteer	LI, NIAID
	F. Ronchese	Visiting Associate	LI, NIAID
	G. Otten	IRTA/Special Volunteer	LI, NIAID
	M. Wroble	Research Technician	LI, NIAID
	L. Hendrix	Research Technician	LI, NIAID
	A. Fox	Research Technician	LI, NIAID

COOPERATING UNITS (if any)

LIG, NIAID (E. Long); LCMI, NIAID (R. Schwartz).

LAB/BRANCH

Laboratory of Immunology

SECTION

Lymphocyte Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

4.5

PROFESSIONAL

3.0

OTHER

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Class II MHC (Ia) gene products play critical roles in a variety of T lymphocyte responses. A combination of immunological, molecular genetic, and biochemical approaches is being used to study the relationship between Ia structure and function.

Ia expression has been examined following transfer of class II genes into L cells. These experiments have shown that the efficiency of cell-surface Ia expression is controlled by both allele-specific and isotype-specific structural features of the α and β chains. There are strong quantitative preferences in the formation and transport of certain heterodimers, explaining the phenotype of cells expressing multiple class II MHC α and β chains. *In vitro* mutagenesis studies have mapped two major regions within the Ia molecule that play key roles in controlling the conformation of Ia molecules required for their transport to the cell membrane.

Analysis of various recombinant Ia molecules with monoclonal antibodies has revealed an unexpected asymmetry in the conformational flexibility of the postulated α -helical regions of the polymorphic Ia domains. Site-directed mutagenesis studies have mapped the precise role of polymorphic residues of the I-E molecule with respect to T cell receptor interaction or peptide antigen binding.

The cell biology of antigen processing and presentation by MHC molecules has been studied and a special role for the endosome/trans-Golgi low pH compartments demonstrated. The rules controlling class I vs class II MHC antigen presentation have been examined using artificially processed soluble protein antigens, with the data indicating that the way antigen enters a cell may determine the MHC molecule used for presentation to T cells.

These studies are providing new insight into the rules governing T cell co-recognition of antigen and MHC molecules, and the biochemistry and cell biology of protein multimer synthesis and transport.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetic Analysis of Lymphocyte Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	David H. Margulies	Section Head	LI, NIAID
Others:	Lisa Boyd	Chemist	LI, NIAID
	Jonathan Schneck	Medical Staff Fellow	LI, NIAID
	Rosemarie Hunziker	Guest Worker	LI, NIAID
	Steven Kozlowski	Medical Staff Fellow	LI, NIAID

COOPERATING UNITS (if any)

LI, NIAID (J. Inman, R. Germain); IB, NCI (T. Munitz, A. Singer); MB, NCI (H. Takahashi, J. Berzofsky); BRB, NIAID (D. Handy, J. Coligan, L. Maloy, R. Anderson); LMB, NIDDK (E. Padlan, D. Davies); NI, NINCDS (W. Biddison).

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

5.0

PROFESSIONAL:

4.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The ongoing focus of the laboratory is to understand in detail the structure/function relationships of cell surface molecules involved in the immune response. In particular, we have made significant progress over the past year in several areas: A. generating a panel of molecularly engineered soluble counterparts of the murine class I major histocompatibility antigens H-2K^b, H-2D^d, H-2L^d, H-2K^k, H-2K^{bm6}, H-2K^{bm8}, H-2K^{bm10}, and the human class I MHC molecule HLA-A2; B. using these soluble molecules to study the biochemical requirements for activation of allospecific T lymphocytes; C. using these soluble molecules to estimate the affinity of an H-2K^b-reactive T cell receptor for the H-2K^b molecule; D. identifying a region of the class I molecule that appears to be a major T cell receptor binding site; E. studying the binding of antigenic viral peptides to purified soluble MHC class I molecules; F. producing transgenic mice that express a soluble counterpart of the soluble H-2D^d molecule to study fundamental questions of the generation of T cell tolerance and education; G. developing a strategy for the production of soluble counterparts of membrane molecules allowing dimeric production and simple purification.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetic Analysis of T Cell Receptor Structure and Repertoire

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	R. N. Germain	Section Head	LI, NIAID
Others:	T. Saito	Visiting Associate	LI, NIAID
	C. Layet	Visiting Fellow	LI, NIAID
	M. Okajima	IRTA	LI, NIAID
	K. Hsu	Research Technician	LI, NIAID

COOPERATING UNITS (if any)

BRMP, NCI (A. Kruisbeck, J. Ashwell); LIR, NIAID (C. Lane).

LAB/BRANCH

Laboratory of Immunology

SECTION

Lymphocyte Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

4.5

PROFESSIONAL

3.5

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

T lymphocytes divide and differentiate when they receive an appropriate activation signal. This signal can be provided by a clonally-distributed, specific antigen receptor (Ti-T3), or through certain broadly distributed cell membrane structures. Molecular biological and *in vitro* cell culture techniques have been used to investigate the expression of the genes encoding these activation structures, and to study the relationship between structure and function.

We have shown by DNA-mediated gene transfer that the two defined T cell receptor heterodimers assemble in a mutually exclusive manner. Thus, in cells possessing all four chains ($\alpha, \beta, \gamma, \delta$) after transfection, only $\alpha\beta$ and $\gamma\delta$ dimers were found associated with CD3 on the T cell membrane. In such cells, the two dimers can compete for limiting CD3, leading to rapid degradation of the non-CD3 associated molecules.

Analysis of cells possessing competing functional α and β chains unexpectedly revealed striking asymmetries in the efficiency of expression of certain chain combinations. The variation between two given combinations could be as great as 20x. Much of this difference can be attributed to the germline V β segment, implying that this region interacts with the α chain in a structurally constrained manner. This observation suggests that the T cell repertoire may be limited in part by the failure of certain $\alpha\beta$ combinations to yield enough surface receptor for function.

Experiments with a CD3 negative murine hybridoma made CD3 positive by TCR gene transfection raised questions about the currently accepted model of receptor mediated signalling. Thus, this reconstituted cell regained the ability to be activated by anti-Thy1 or anti-Ly6 monoclonal antibodies, but its response (measured as IL2 secretion) occurred in the absence of detectable changes in the intracellular $[Ca^{2+}]$ or generation of phosphoinositides. These findings suggest that other second signals may play a critical role in gene activation following receptor occupancy.

These studies will enhance our knowledge of which cell membrane molecules are involved in triggering T cells to exert regulatory and effector function, and our understanding of the structural basis for specific T cell responses to antigen.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00425-04 LI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Lymphocyte Physiology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T.M. Chused	Section Head	LI, NIAID
Others:	Y.A. Ishida	Visiting Fellow	LI, NIAID
	E.M. Brown	Biologist	LI, NIAID

COOPERATING UNITS (if any)

LI, NIAID (W.E. Paul)

LAB/BRANCH

Laboratory of Immunology

SECTION

Transmembrane Signalling Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|--------------------------------------|
| ☐ (a) Human subjects | ☐ (b) Human tissues | ☐ (c) Neither |
| ☐ (a1) Minors | | |
| ☐ (a2) Interviews | | |

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The process of signal transduction across the lymphocyte plasma membrane is under investigation. Novel fluorescent probes of physiologic parameters such as membrane potential, intracellular free ionized calcium, and intracellular pH, in conjunction with the high sensitivity and single cell resolution of flow cytometry, are being utilized. These studies have revealed extensive "feed-forward" and "feed-back" regulatory relationships between ion channel opening, membrane potential, activity of the calcium pump, and rate of phosphatidyl inositol turnover. These mechanisms differ in the T, B and monocyte/granulocyte lineages.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00427-04 LI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antigen-Specific and Antigen-Nonspecific Cellular Cytotoxicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Michail V. Sitkovsky	Visiting Scientist	LI, NIAID
Others:	Hajime Takayama	Visiting Fellow	LI, NIAID
	Guido Trenn	Guest Worker	LI, NIAID
	Rolf Taffs	Staff Fellow	LI, NIAID
	David H. Margulies	Section Head	LI, NIAID
	Randall Kincaide	Section Head	NIAAA
	Anne Fortier	Guest Worker	LI, NIAID
	William Humphrey	Technical Assistant	LI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

6

PROFESSIONAL:

5

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

To identify proteins (enzymes) involved in T cell-receptor (TCR)-triggered mechano-biochemical activities of cytotoxic T-lymphocytes (CTL), we study the functional organization of cell surface receptors and underlying membrane matrix/cytoskeleton, as well as biochemical events that follow occupation of functionally important surface proteins by appropriate crosslinking ligands, antigens, or mAb. Biochemistry of CTL activation: We developed a novel functional assay (granule exocytosis assay) for CTL activation and characterized activating and inhibitory biochemical pathways. These pathways are implicated in "ON" and "OFF" signalling which forms a basis for cyclic activities of CTL. Protein kinase C and calmodulin-binding proteins (CaM-BP) are part of TCR-triggered activating pathway, while cAMP-dependent protein kinase which counteracts both early and late events of activating pathway, represents the key enzyme of inhibiting pathway. The biochemical studies of T-cells suggested that CaM-dependent phosphatase (calcineurin) is the main beneficiary of TCR-induced increases in intracellular Ca^{++} and pointed to the dephosphorylation reactions as the major response to TCR crosslinking. Plasma membrane organization: During functional studies of organization of surface proteins we came to the conclusion that inhibition of CTL activation by anti-CD8 mAb is most likely explained by physical proximity of CD8 with TCR. However, near-neighbor analysis studies with chemical cross-linking reagents unexpectedly revealed that CD8 is most easily crosslinked to class I MHC proteins on the surface of CTL. The experiments designed to isolate and characterize functional domains in CTL plasma membrane suggest the existence of a subset of surface proteins which are associated with membrane matrix and demonstrated TCR-dependent changes in their organization. Immunopharmacological implications: A novel strategy is suggested for manipulation of immune response by taking advantage of newly-found knowledge of biochemistry of lymphocyte activation. Non-tumor promoting protein kinase C activators (e.g. compounds of Bryostatin family) and antagonists of cAMP (e.g. Rp-cAMPS) are proposed to be used as immunoenhancing drugs.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

B Cell Stimulatory Factor-1 (BSF-1)/ Interleukin-4 (IL-4)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	William E. Paul	Chief	LI/NIAID
Others:	Junichi Ohara	Visiting Scientist	LI/NIAID
	Clifford Snapper	Medical Staff Fellow	LI/NIAID
	Christian Peschel	Visiting Associate	LI/NIAID
	Graham LeGros	Visiting Fellow	LI/NIAID
	Ulus Atasoy	NRSA Fellow	LI/NIAID
	Marshall Plaut	Guest Researcher	LI/NIAID
	Dan Conrad	Guest Researcher	LI/NIAID

COOPERATING UNITS (if any)

NCI (J. Pierce); Uniformed Services Univ. Health Sciences (F. Finkelman, I. Katrona);
Dept Agriculture (J. Urban); Harvard School of Public Health (L. Glimcher).

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

5.2

PROFESSIONAL:

3.2

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Interleukin-4 (IL-4)/B cell stimulatory factor-1 is a T cell derived lymphokine with potent effects on a wide variety of hematopoietic and on some non-hematopoietic cells. It costimulates B cell DNA synthesis, acting with anti-immunoglobulin antibody to cause B cell division; it upregulates expression of class II MHC molecules and of FcεRII (CD23 molecules) on resting B cells. Together with lipopolysaccharide, it strikingly enhances the production of IgG1 and of IgE and causes B cells to express Thy-1 on their membranes. The effect of IL-4 in regulating IgG1 and IgE synthesis and Thy-1 expression is also observed in B cells stimulated in cognate interactions with T cells that produce IL-4. Moreover, anti-IL-4 treatment of mice innoculated with live Nippostrongylus brasiliensis larvae causes a virtual complete inhibition of the induction of polyclonal IgE responses. Anti-IL-4 also inhibits IgE responses in other models of primary and secondary IgE responses. These results indicate that IL-4 is an essential factor in the in vivo regulation of IgE production.

Although T cells are normally thought of as the principal if not the sole source of IL-3, IL-4 and IL-5, we have now shown that non-transformed factor-dependent mast cell lines stimulated with calcium ionophores secrete IL-3 and IL-4 and express substantial amounts of mRNA for IL-3, IL-4, and IL-5. Mast cell lines release IL-3 and express mRNA for IL-3, IL-4 and IL-5 when their Fcε receptors are cross-linked. These results suggest that mast cells may participate in allergic inflammation not only through the release of vasoactive amines but also through the production of factors that regulate the growth of mast cells and eosinophils and that cause switching to the production of IgE.

Efforts to characterize the IL-4 receptor and to clone the IL-4 receptor gene are in progress. In addition, a cell line has been obtained, CT.4S, that provides a specific and sensitive bioassay for IL-4. IL-4 has also been expressed in the baculovirus system, providing a source for very substantial amounts of the recombinant product.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1987 to September 30, 1988

*TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of T Lymphocyte Responses to HIV Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: R. N. Germain

Section Head

LI, NIAID

COOPERATING UNITS (if any)

LVD, NIAID (B. Moss); MC, NCI (H. Takahashi, J. Berzofsky).

LAB/BRANCH

Laboratory of Immunology

SECTION

Lymphocyte Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

.25

PROFESSIONAL:

.25

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

To develop effective therapeutic and vaccine strategies to combat infection with HIV-1, the cause of AIDS, it is essential to understand the cell-mediated immune response to this infectious agent. Towards this end, model systems for immunization with HIV proteins are being devised and used to test the ability of these proteins to stimulate various subsets of T lymphocytes.

Initial experiments have utilized a mouse model. Animals were immunized with recombinant vaccinia virus containing an expressible form of the env gene of the IIIB strain of HIV-1, encoding the gp160 molecule. Lymphoid cells from such immunized mice were restimulated *in vitro* either with vaccinia recombinant virus infected cells or transfected cells expressing the gp160 protein. H-2^d T cells from such restimulated cultures were found to contain cytotoxic T lymphocytes (CTL) with specificity for the gp160 protein in the context of H-2D^d. No such responses were observed with cells from H-2^k mice.

Synthetic peptides corresponding to segments of the gp160 molecule were used to map the sites being recognized by these CTL. Only a single major recognition site was identified that appeared to be the target of essentially all CTL elicited under these conditions. This site was in an area of extensive sequence variation among isolates of HIV-1.

These data indicate that there may be significant effects of MHC gene polymorphism on the response to HIV proteins and that the immunodominant regions may be subject to strong selection *in vivo*, resulting in viral escape from immune recognition following immunization with antigen corresponding to a single sequence. Additional studies are underway to extend these observations to other HIV proteins and to responses by human T cells.

LABORATORY OF IMMUNOPATHOLOGY
1988 Annual Report
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PHS-NIH
SUMMARY REPORT

ANNUAL REPORT OF THE LABORATORY OF IMMUNOPATHOLOGY, NIAID
October 1, 1987 to September 30, 1988

Herbert C. Morse III, M.D.
Chief, Laboratory of Immunopathology

Programs in the Laboratory of Immunopathology continue to focus on host-virus interactions, with particular emphasis on murine retroviruses (MuLV) and papovaviruses, and development and function of the hematopoietic system. The viruses are studied for the mechanisms by which they induce neoplastic transformation, immunodeficiency or other disorders using a wide variety of techniques in tissue culture virology, molecular biology, protein chemistry, cell biology and cellular immunology. Different host species are studied for genetic determinants of resistance and susceptibility to disease and immunologic responses that contribute to the development of these phenotypes. Patterns of normal hematopoietic differentiation are evaluated to understand how particular cell lineages are targeted for transformation by specific viruses, and how these patterns are altered by virus infections or mutations that result in development of autoimmunity. Studies of normal cells are complemented by analyses of tumor cells transformed in vitro or in vivo by a variety of MuLV or DNA viruses. Studies of naturally-occurring and molecularly engineered recombinant viruses have provided fundamental insights into the nature of specific viral sequences critical to disease induction. Finally, analyses of acutely transforming viruses containing oncogenes have been used to determine how aberrant expression of cellular genes affects the biology of different cells to produce the transformed phenotype.

Over the last four years, members of LIP have been studying the pathogenesis of a retrovirus-induced immunodeficiency syndrome of mice, termed MAIDS. During the last year, considerable progress has been made in understanding the characteristics of the etiologic viruses, the effects of infection on the immune system, and genetic determinants of resistance to disease. The virus preparation causing MAIDS was found earlier to contain a mixture of ecotropic and mink cell focus-inducing (MCF) MuLV. We have shown that biologically cloned infectious viruses belonging to either class do not induce the major manifestations of mouse AIDS - lymphadenopathy, polyclonal B cell activation or immunosuppression. We now have biologic and molecular genetic evidence that a deleted, replication-defective MuLV genome is regularly associated with rapid induction of disease and that, pseudotyped by ecotropic MuLV, it plays a major role in the pathogenesis of MAIDS (Chattopadhyay, Makino, Morse, Hartley).

The parallels between MAIDS and HIV-induced abnormalities in man have been greatly extended. We demonstrated that the late stages of MAIDS are characterized by the development of B cell lineage lymphomas that evolve progressively from polyclonally activated B cells (Klinken, Fredrickson, Yetter, Hartley, Morse). The early stage of B cell activation was shown to result in the production of many types of autoantibodies (Klinman, Morse). Studies of cytotoxic T lymphocyte (CTL) responses to "self + x" and alloantigens showed that the former responses were lost before the latter following infection with LP-BM5 MuLV, but that both could be restored to normal by addition of exogenous IL-2 during the early phases of disease (Yetter, Shearer, Via, Miller, Morse). Biologic and molecular studies of IL-2 showed that expression was not stimulated by infection and that the ability to produce IL-2 was lost with time after infection (Yetter, Pitha, Morse). In contrast, infection with LP-BM5 MuLV stimulated high level expression of gamma interferon while inhibiting the ability of mice to express either alpha or beta interferon (Pitha, Biegel, Yetter, Morse). Finally, mice infected with LP-BM5 MuLV were shown to exhibit impaired natural killer cell and activated killer cell functions, again reflecting abnormalities consequent to infection with HIV (Wunderlich, Yetter, Morse).

Recent studies have suggested that genes of the major histocompatibility complex (MHC) may determine the progression to disease of individuals infected with HIV. We have shown that the MHC of the mouse also contains a major determinant of resistance of certain inbred mouse strains to MAIDS. C57BL mice (Fv-1^b) are highly sensitive to induction of MAIDS by LP-BM5 MuLV while several other Fv-1^b strains, including BALB/c and A, show marked resistance. The major control of these differences in susceptibility was found to be exerted by genes in the H-2 region; haplotypes b, r and j confer sensitivity; a and d are resistant; and k gives an unusual intermediate pattern. Tests of mice recombinant in H-2 suggest a predominant influence of genes at or near H-2D (Makino, Yetter, Hartley, Morse). It is noteworthy that recent studies of CTL responses of mice to recombinant HIV gp120 show that the dominant epitope of the molecule is H-2D^d restricted.

Based on these results, it should now be possible to obtain molecular clones of the etiologic virus, to determine how its structure contributes to induction of this unique syndrome, and to study the immunologic mechanisms that mediate resistance to disease.

While particular emphasis has been placed on analyses of the MAIDS model system - a major component of the efforts of the Viral Oncology and Viral and Cellular Immunology Sections - a number of other important findings highlight the studies performed in the Laboratory of Immunopathology over the last year. They include the following observations.

Sequences in three portions of retroviral LTR interact to specify the target cells of Moloney and Friend MuLVs. In the enhancer regions of these two viruses, the direct repeat sequences are identical in a central core region but differ either side of this core; differences also occur in a short GC-rich segment with promoter activity immediately downstream of the direct repeat. When tested separately and in combination in recombinant virus constructs, all 3 regions of diversity were found to influence disease-inducing phenotype. Combinations of 2 segments derived from 1 LTR, especially 2 halves of the direct repeat, were much more effective than single segments in specifying lymphoma or erythroleukemia (Hartley, Hopkins).

Latency of lymphoma or erythroleukemia induced by Moloney/Friend LTR recombinant viruses also is influenced by sequences in both halves of the direct repeat. Latent periods for development of disease were extended in mice inoculated with recombinant genomes in which the 5' half of each copy of the direct repeat was derived from Moloney and the 3' halves were from Friend (Hartley, Hopkins).

Viral gene inhibition of class I major histocompatibility antigen expression: not a general mechanism governing the tumorigenicity of adenovirus type 2-, adenovirus type 12-, and simian virus 40-transformed Syrian hamster cells. The association between the level of class I major histocompatibility (MHC) antigen expression and the tumor inducing capacity has been determined for cells from 31 lines of adenovirus type 2 (Ad2)-, Ad12-, and simian virus 40 (SV40)-transformed hamster cells. These cells range from nontumorigenic to highly tumorigenic in both syngeneic and allogeneic adult hamsters. Nontumorigenic Ad2-transformed cells expressed either high levels or low levels of class I MHC antigens. Highly tumorigenic SV40-transformed cells expressed very high levels of class I MHC antigens. Ad12-transformed cells that expressed low levels of class I MHC antigens produced tumors much less efficiently in allogeneic adult hamsters than in syngeneic adult hamsters and produced tumors with about the same efficiency in immunoimmature newborns and immunocompetent syngeneic adult hamsters. Thus the expression of either high levels or low levels of class I MHC antigens is, at most, a minor factor in the differences observed among these adenovirus- and SV40-transformed cells in their tumor-inducing capacity in naive, immunocompetent hamsters. (Haddada, Sogn, Coligan, Carbone, Dixon, Levine, Lewis)

Ad12 transformed BALB/c cells expressing low levels of class I MHC proteins are rejected as allografts. It has been proposed that Ad12 Ela-gene induced suppression of class I major histocompatibility (MHC) proteins on the surface of Ad12 transformed cells is causally related to their tumorigenicity. Evaluation of this proposal has produced conflicting results. In a continuing effort to evaluate the role of class I MHC protein expression in the tumorigenicity of Ad transformed cells, we

developed and characterized three lines of Ad12 transformed BALB/c mouse embryo cells (Ad12ME1, Ad12ME2, Ad12ME3). These lines contain Ad12 DNA and express Ad12 T proteins. Cells from each line expressed low levels of class I MHC mRNA and class I cell-surface proteins, and they induced tumors in syngeneic adult BALB/c mice. Cells from each of these 3 lines fail to produce tumors when injected into allogeneic mice or in mice that express variable recombinant MHC haplotypes between the syngeneic and allogeneic mouse strains. These data suggest that, in spite of the low levels of class I MHC proteins expressed by these cells, they can be recognized and rejected as allografts by H-2 divergent mice. (Soddu, Haddada, Sogn, Levine, Lewis).

In vivo evolution of adenovirus 2 transformed cell virulence is associated with altered E1A gene function. Cells transformed by nononcogenic Ad2 can be adapted by serial passage in immunologically incompetent animals to produce tumors in fully immunocompetent syngeneic adult animals. We compared the functions of the Ad2 E1A genes in adult adapted Ad2 transformed cells with their functions in the nontumorigenic parental cells. The Ad2 transformed cells that were selected for increased tumorigenicity retained parental patterns of viral gene integration and methylation and expressed Ad2 E1A proteins but exhibited altered E1A functions as suggested by markedly decreased susceptibility to NK lysis and the inability to support the replication of an Ad5 E1A mutant that replicated to high titers in the parental cells. These data suggest that disruption of cellular pathways induced or altered by E1A functions during tumor cell adaptation results in an increased degree of tumor cell virulence. (Lewis)

Peculiar persistence and expression of viral genomes in adenovirus 5 transformed BALB/c mouse cells. Transformation by Ad5 has been mostly studied in rat and hamster cells. To expand the Ad5 transformation model, we have begun to study several lines of Ad5 transformed, BALB/c mouse embryo cells. The Ad5 genome in these cells is expressed in unusual ways compared to viral gene expression in Ad5 transformed rat and hamster cells. Cells from 1 line express both Ad5 DNA and RNA and proteins. Cells from a second line contain Ad5 DNA and express Ad5 mRNA but not Ad5 protein is not detected in these cells. Cells from a third line contain Ad5 DNA, but fail to express Ad mRNA. Cells from a fourth line appear similar to cells from the other lines, but do not contain Ad5 DNA. These results suggest that, in certain instances in the Ad5-BALB/c mouse cell system, Ad5 may only initiate transformation and may be dispensable once the critical event has occurred. Persistence expression of Ad5 viral genes in mouse cells may not be related to their tumor inducing capacity in nude mice (Carbone, Chattopadhyay, Lewis).

Effects of deletions in the small t region of SV40 genome: induction of true histiocytic lymphomas distant from the site of virus inoculation in Syrian hamsters. To evaluate the various phenotypes that may be expressed by DNA virus transformed cells

that are tumorigenic in immunocompetent syngeneic adults, we have started to characterize cells from an unusual type of reticulum cell sarcomas induced in hamsters by certain SV40 mutants. In contrast to tumors induced by wild type virus, these tumors develop at sites distant from the site of inoculation.

Morphological and functional analyses indicate that these tumor cells are derived from MAC-2⁺ macrophages. Thus, these mutations in the SV40 genome appear to permit the virus to transform cells that are distant from the site of virus inoculation; the cells transformed at this distant site are of a specific lineage, MAC-2⁺ peritoneal macrophages. This specific tropism may reflect a unique characteristic of MAC-2⁺ cells or their precursors that renders these cells susceptible to SV40 mutants that are otherwise restricted in the range of cells which they can transform (Carbone, Lewis, Matthews, Levine, Dixon).

A new oncogene, v-cbl, that induces pre-B cell lymphomas. The murine Cas NS-1 retrovirus transforms fibroblasts in vitro and induces pre-B cell lymphomas in vivo. This virus arose in a mouse that developed a pre-B cell lymphoma following neonatal infection with the Cas-Br-M ecotropic virus. Molecular cloning showed it was generated from Cas-Br-M by sequential recombinantions involving endogenous retroviral sequences and a cellular oncogene. The oncogene sequence shows no homology with known oncogenes but some similarity to the yeast transcriptional activator GCN4 (Langdon, Hartley, Klinken, Ruscetti, Morse).

Cellular sequences related to v-cbl are present on two different chromosomes. Probes derived from the v-cbl oncogene were used to map related cellular sequences through analyses of somatic cell hybrids and backcross mice from an interspecific cross. v-cbl-related sequences were localized to chromosome 6 in linkage to the kappa locus (c-cbl-1) and on chromosome 9 in close linkage to Thy-1 (c-cbl-2) (Regnier, Kozak, Kingsley, Langdon, Morse).

Administrative Changes. Dr. Marjorie Shapiro joined the Laboratory on an Intramural Research Training Appointment and Dr. Masahiko Makino began his studies on a Fogarty Fellowship. Dr. Robert A. Yetter left the Laboratory to join the Veterans Administration Research Center in Baltimore, MD. Drs. Daniel Regnier and Silvia Soddu joined the Laboratory as Guest Researchers.

Honors and Awards

Public Health Service Distinguished Service Award - Janet W. Hartley.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 201 AI 00013-25 LIP
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters per line. Title must fit on one line between the borders.) Immunobiology and Pathogenesis of DNA Virus Infections		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI : A. M. Lewis, Jr. Hd, Viral Pathogenesis Section LIP, NIAID Others: M. Carbone Visiting Fellow LIP, NIAID S. Soddu Guest Researcher LIP, NIAID J. A. Sogn Senior Investigator LIG, NIAID J. Coligan Senior Investigator LIG, NIAID A. S. Levine Scientific Director OSD, NICHD C. T. Patch Senior Investigator OSD, NICHD K. Dixon Senior Investigator OSD, NICHD		
COOPERATING UNITS (if any) National Jewish Center for Immunology and Respiratory Medicine, Denver, CO (J. L. Cook).		
LAB BRANCH Laboratory of Immunopathology		
SECTION Viral Pathogenesis Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 4.0	PROFESSIONAL 1.0	OTHER 4.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) We have shown that the E1A gene of nononcogenic Ad2 and Ad5 induces susceptibility to NK cell and macrophage mediated lysis in transformed rodent cells and that the expression of high levels of susceptibility correlates with transformed cell tumor inducing capacity. Adaptation of Ad2 transformed cells to produce tumors in tumor resistant syngeneic adult hamsters by serial passage in newborns results in cells that are resistant to NK and macrophage killing and whose E1A gene function is disrupted to the extent that these cells will not support replication of Ad2 E1A mutants. We have also found that Ad12 transformed hamsters cells that have low levels of class I MHC antigens on their surfaces are generally nontumorigenic in allogenic hamsters and mice and are highly immunogenic in bioassays for CTL mediated detection of Ad12 transplantation antigens. Our Ad12 transformed BALB/c mouse cells (haplotype H-2 ^d) produce tumors in BALB/c and DBA-2 mice (haplotype H-2 ^d) but fail to produce tumors in mice with recombinant haplotypes (H2-K ^d , H-2D ^b , H-2L ^b or H-2K ^b , H-2D ^d , H-2L ^d); thus these cells can be rejected as allografts by H-2 divergent mice in spite of their low levels of class I MHC antigen expression. Several Ad5 transformed BALB/c mouse cell lines that we have developed express peculiar patterns of expression of the Ad5 genome. Some of the cells retain Ad5 DNA, express Ad5 mRNA and Ad5 early proteins. Others retain Ad5 DNA but fail to express Ad5 mRNA. One line that is morphologically similar to the others contains no Ad5 DNA. These data suggest that persistence of the Ad5 genome and expression of viral protein may not be essential to maintaining the neoplastic phenotype of Ad5 transformed BALB/c mouse cells.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00135-14 LIP																																
PERIOD COVERED October 1, 1987 to September 30, 1988																																		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Properties of Immunoglobulin Secreting Cells																																		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI :</td> <td style="width: 45%;">H. C. Morse III</td> <td style="width: 20%;">Chief</td> <td style="width: 20%;">LIP, NIAID</td> </tr> <tr> <td>Others:</td> <td>W. F. Davidson</td> <td>Visiting Scientist</td> <td>LG, NCI</td> </tr> <tr> <td></td> <td>J. W. Hartley</td> <td>Senior Investigator</td> <td>LIP, NIAID</td> </tr> <tr> <td></td> <td>T. N. Fredrickson</td> <td>Research Microbiologist</td> <td>LIP, NIAID</td> </tr> <tr> <td></td> <td>K. L. Holmes</td> <td>Sr. Staff Fellow</td> <td>LIP, NIAID</td> </tr> <tr> <td></td> <td>S. P. Klinken</td> <td>Visiting Fellow</td> <td>LIP, NIAID</td> </tr> <tr> <td></td> <td>M. A. Principato</td> <td>Guest Researcher</td> <td>LIP, NIAID</td> </tr> <tr> <td></td> <td>U. R. Rapp</td> <td>Senior Investigator</td> <td>LVC, NCI</td> </tr> </table>			PI :	H. C. Morse III	Chief	LIP, NIAID	Others:	W. F. Davidson	Visiting Scientist	LG, NCI		J. W. Hartley	Senior Investigator	LIP, NIAID		T. N. Fredrickson	Research Microbiologist	LIP, NIAID		K. L. Holmes	Sr. Staff Fellow	LIP, NIAID		S. P. Klinken	Visiting Fellow	LIP, NIAID		M. A. Principato	Guest Researcher	LIP, NIAID		U. R. Rapp	Senior Investigator	LVC, NCI
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LAB/BRANCH Laboratory of Immunopathology																																		
SECTION Virology and Cellular Immunology Section																																		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892																																		
TOTAL MAN-YEARS. 3.0	PROFESSIONAL: 2.0	OTHER: 1.0																																
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SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Cells related to the B lymphocyte lineage of mice were studied to determine their developmental relationships to other hematopoietic cell types and the contributions of oncogenes to tumor induction. B cell development: a wide variety of hematopoietic neoplasms were studied for the organization of immunoglobulin and T cell receptor genes to determine lineage relationships based on the activity of recombinases. Rearrangements of receptor genes were detected in T cell=B cell >> myeloid tumors but not in erythroleukemias, suggesting an early deviation of erythroid progenitors from precursors for the other lineages. Close relations between the B cell and myeloid lineages are also suggested by the demonstration that B cell progenitor lines can differentiate to yield mature macrophages. <u>Oncogenes</u>: a new oncogene that induces pre-B cell <u>lymphomas in vivo</u> and transforms fibroblasts <u>in vitro</u> was cloned and sequenced. The sequence was unrelated to that of any previously described oncogene and it has been named <u>v-cbl</u>. Cellular sequences related to <u>v-cbl</u> were mapped to mouse chromosomes 6 and 9 and were designated <u>c-cbl-1</u> and <u>c-cbl-2</u>. Mice inoculated with retroviruses containing <u>v-myc</u> were found to develop clonal <u>pancreatic acinar cell carcinomas</u> that could be transplanted and established as <u>in vitro</u> cell lines. In contrast, mice inoculated with retroviruses containing <u>c-myc</u> developed myeloid tumors.																																		

Other Investigators:

M. Potter	Chief	LG, NCI
J. F. Mushinski	Investigator	LG, NCI
J. H. Pierce	Investigator	LCMB, NCI
W. Y. Langdon	Investigator	Walter and Eliza Hall Institute, Melbourne, Australia
D. Regnier	Guest Researcher	LIP, NIAID
M. Shapiro	IRTA	LIP, NIAID

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00138-14 LIP
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Viruses and Immune Response		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI :	H. C. Morse III Chief	LIP, NIAID
Others:	J. W. Hartley Senior Investigator K. L. Holmes Senior Staff Fellow T. N. Fredrickson Research Microbiologist R. M. L. Buller Senior Staff Fellow A. Singer Senior Investigator	LIP, NIAID LIP, NIAID LIP, NIAID LVD, NIAID I, NCI
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Immunopathology		
SECTION Virology and Cellular Immunology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5
CHECK APPROPRIATE BOX(ES)		
☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☐ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
Both the humoral and cellular arms of the immune response contribute to control of <u>virus infections</u>. <u>Ectromelia</u>, an orthopox virus infection of mice has been selected as a model system for studying host-virus interactions. It was found that Ly-2⁺ T cells are sufficient for <u>in vivo</u> resistance to this virus and that <u>in vitro</u> secondary responses to vaccinia are effected by interactions between Ly-2⁺ helper and effector cells. <u>Interleukin-2</u> appears to mediate the interactions between these subsets of T cells.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 AI 00205-08
LIP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (40 characters or less. Title must fit on one line between the borders.)

Abnormalities of T and B Lymphocytes of Autoimmune Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI	:	H. C. Morse III	Chief	LIP, NIAID
Others:		W. F. Davidson	Visiting Scientist	LG, NCI
		C. Calkins	Guest Researcher	LIP, NIAID
		M. Seldin	Investigator	ARB, NIAMS
		A. D. Steinberg	Investigator	ARB, NIAMS

COOPERATING UNITS (if any)

LABORATORY

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

1.0

PROFESSIONAL

0.75

OTHER

0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Genetic and environmental factors interact in the development of autoimmune disease in mouse and man. In the mouse, two autosomal recessive mutations, lpr and gld, contribute to syndromes characterized by production of lymphoproliferation and autoantibodies. To evaluate mechanisms by which these mutations result in autoimmunity, we have initiated attempts to molecularly clone the gld locus and have studied lymphokine production by the abnormal Thy-1⁺, Ly-2⁻, Ly-4⁻ cells that accumulate in tissues of the mice. To facilitate cloning gld, a molecular map of distal mouse chromosome 1 is being generated and gld has been shown to map in close proximity to the At-3 locus.

Studies of lymphokine-specific mRNA species in the abnormal T cells of lpr and gld mice have shown that they do not express significant levels of message for IL-6, IL-5, IL-4, TNF, or GM-CSF. Neuroleukin was expressed in both cell types at levels equivalent to normal T cells. Further studies are required to determine how these unusual cells participate in the development of autoimmunity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00284-07 LIP																								
PERIOD COVERED October 1, 1987 to September 30, 1988																										
TITLE OF PROJECT (60 characters or less. Title must fit on one line between the borders) Characterization of Pathogenic Murine Leukemia Viruses																										
PRINCIPAL INVESTIGATOR (List either professional personnel below the Principal Investigator, if Name, title, laboratory, and institution are listed) PI: J. W. Hartley, M.D., Viral Oncology Section, LIP, NIAID Others: <table style="width: 100%; border: none;"> <tr> <td style="width: 35%;">H. C. Morse III</td> <td style="width: 35%;">Chief</td> <td style="width: 30%;">LIP, NIAID</td> </tr> <tr> <td>T. N. Fredrickson</td> <td>Research Microbiologist</td> <td>LIP, NIAID</td> </tr> <tr> <td>R. A. Yetter</td> <td>Guest Researcher</td> <td>LIP, NIAID</td> </tr> <tr> <td>K. L. Holmes</td> <td>Senior Staff Fellow</td> <td>LIP, NIAID</td> </tr> <tr> <td>S. K. Chattopadhyay</td> <td>Expert</td> <td>LIP, NIAID</td> </tr> <tr> <td>M. Makino</td> <td>Visiting Fellow</td> <td>LIP, NIAID</td> </tr> <tr> <td>R. Repaske</td> <td>Senior Investigator</td> <td>LMM, NIAID</td> </tr> <tr> <td>S. Ruscetti</td> <td>Investigator</td> <td>NCI</td> </tr> </table>			H. C. Morse III	Chief	LIP, NIAID	T. N. Fredrickson	Research Microbiologist	LIP, NIAID	R. A. Yetter	Guest Researcher	LIP, NIAID	K. L. Holmes	Senior Staff Fellow	LIP, NIAID	S. K. Chattopadhyay	Expert	LIP, NIAID	M. Makino	Visiting Fellow	LIP, NIAID	R. Repaske	Senior Investigator	LMM, NIAID	S. Ruscetti	Investigator	NCI
H. C. Morse III	Chief	LIP, NIAID																								
T. N. Fredrickson	Research Microbiologist	LIP, NIAID																								
R. A. Yetter	Guest Researcher	LIP, NIAID																								
K. L. Holmes	Senior Staff Fellow	LIP, NIAID																								
S. K. Chattopadhyay	Expert	LIP, NIAID																								
M. Makino	Visiting Fellow	LIP, NIAID																								
R. Repaske	Senior Investigator	LMM, NIAID																								
S. Ruscetti	Investigator	NCI																								
COOPERATING INSTITUTIONS (List all institutions participating in the project) Massachusetts Inst. of Technology, Cambridge, MA (N. Hopkins); Georgetown Univ., Washington, DC (B. Baroudi); Univ. of Massachusetts, Worcester, MA (C. Holland)																										
LABORATORY Laboratory of Immunopathology																										
SECTION Viral Oncology Section																										
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892																										
MAN-YEARS 3.3	PROFESSIONAL 1.1	OTHER 2.2																								
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews																										
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) The general objective of this project is the isolation, biological, and molecular characterization of C-type murine leukemia viruses (MuLVs). Major focus has been on the molecular genetics of the pathogenesis in NFS mice of Moloney (Mol) MuLV-induced lymphoma and Friend (Fr) MuLV-induced erythroleukemia and of lymphomagenesis by a helper-independent MCF virus, NS-6 MCF; and biological and molecular characterization of the LP BM5 viruses involved in induction of mouse AIDS. Studies with Mol and Fr recombinant genomes have established that the major determinant of disease specificity lies within that portion of the viral LTR comprising direct repeat sequences identified with transcriptional enhancer signals. Using recombinants constructed between Fr and Mol within this segment disease specificity was found to be encoded throughout the region (in the 5' and 3' halves of the direct repeat and in an adjacent downstream GC-rich region with enhancer-promoter function). In preparation for molecular genetic studies of NS-6 MCF MuLV the molecularly cloned and sequenced genome has been engineered to permit comparison of LTRs. In efforts to determine which of the components of LP BM5 MuLV mixture, a tissue culture stock known to contain both B-tropic ecotropic and MCF MuLVs, several biologically cloned ecotropic and MCF isolates failed to induce disease, alone or in combination. Virus interference studies and molecular and biological analysis of virus derived from single cell clones of infected cells indicate that disease induction is correlated with a defective genome, 5.0 kb in length, which contains MCF-reactive sequences. Spread of virus is dependent on replication competent virus, the ecotropic MuLV likely being the most efficient helper.																										

NOTICE OF INTRAMURAL RESEARCH PROJECT

C01 AI 00286-07
LIP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Genetic Control of Murine Leukemia Viruses and Virus-Induced Neoplasms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI : J. W. Hartley Hd, Viral Oncology Section LIP, NIAID

Others:	M. Makino	Visiting Fellow	LIP, NIAID
	H. C. Morse III	Laboratory Chief	LIP, NIAID
	T. N. Fredrickson	Guest Researcher	LIP, NIAID
	R. A. Yetter	Guest Researcher	LIP, NIAID

COOPERATING UNITS (if any)

U. of Texas System Cancer Center (F. Becker).

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

...EARS

3.6

PROFESSIONAL

1.75

OTHER

1.8

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|---|
| ☐ (a) Human subjects | ☐ (b) Human tissues | ☒ (c) Neither |
| ☐ (a1) Minors | | |
| ☐ (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our studies of host genes which influence hematopoietic system diseases associated with MuLV infection have been directed toward genetic factors controlling infection and disease induction by the B-tropic MuLV complex known as LP-BM5. Mouse strains most susceptible to the immunodeficiency disease (mouse AIDS or "MAIDS") induced by LP-BM5 are, as would be predicted, of the Fv-1^b genotype, a background permissive to infection by and spread of B-tropic MuLVs. Only certain Fv-1^b strains, however, were found to be sensitive: C57BL and I/St are highly susceptible while other Fv-1^b strains such as BALB/c and A are resistant. Based on tests of selected inbred, recombinant inbred, and H-2 congenic mouse strains, we have determined that the major determinant of sensitivity lies within the MHC region. Haplotypes b, r, and j confer sensitivity, H-2^k strains show partial sensitivity, while H-2^d and H-2^a strains are resistant. In crosses between C57BL (H-2^b) and H-2^d or H-2^a strains, sensitivity appears to be dominant. Further, tests of H-2 congenic and recombinant mice suggest that the major controlling element lies at the D end of H-2. In addition, it appears that certain combinations of H-2 gene elements, or genes outside the H-2 region, can affect the disease phenotype, e.g., the degree or rate of progression. Induction of disease is strongly correlated with replication of both the ecotropic and MCF components of the LP-BM5 mixture.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00465-03 LIP
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Retrovirus-induced Murine Immunodeficiency Syndrome		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI :	H. C. Morse III	Chief LIP, NIAID
Others:	R. A. Yetter	Guest Researcher LIP, NIAID
	J. W. Hartley	Senior Investigator LIP, NIAID
	C. Shearer	Investigator I, NCI
	J. R. Wunderlich	Investigator I, NCI
	K. L. Holmes	Senior Staff Fellow LIP, NIAID
	S. P. Klinken	Visiting Fellow LIP, NIAID
	S. K. Chattopadhyay	Expert LIP, NIAID
COOPERATING UNITS (if any) Medical Biology Institute, LaJolla, CA (D. Mosier); Johns Hopkins Medical School, Baltimore, MD (P. Pitha).		
LAB/BRANCH Laboratory of Immunopathology		
SECTION Virology and Cellular Immunology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
- JAN-YEARS 4.5	PROFESSIONAL 3	OTHER 1.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) We have developed a model system for studying how <u>retroviruses</u> contribute to induction of <u>immunodeficiency</u> . Mice infected with a mixture of murine leukemia viruses (MuLV), termed LP-BM5 MuLV, develop a syndrome characterized by early-onset lymphoproliferation and immunodeficiency and later by enhanced susceptibility to infection and development of B cell lineage lymphomas. The etiologic virus of this syndrome was found to be a replication defective MuLV with an envelope gene related to MCF MuLV. The virus is being molecularly cloned to develop further understanding of its effects on the immune system. The virus-induced disease was shown to be dependent on Ly-4 ⁺ T cells, as mice depleted <u>in vivo</u> of this cell subset do not develop prominent dysfunction of Ly-2 ⁺ T cells or of B cells. Analyses of lymphokines produced by Ly-4 ⁺ T cells showed that infection stimulated high-level expression of <u>gamma interferon</u> , but that alpha and beta interferon could not be induced. By comparison, infected mice did not produce IL-2, IL-3, IL-4, IL-6 or GM CSF. Lack of IL-2 production was associated with deficient <u>cytotoxic T lymphocyte</u> responses and possibly with impaired <u>natural killer cell</u> responses. A consequence of Ly-4 ⁺ T cell activation is the development of polyclonal B cell activation that precedes the appearance of oligoclonal B cell proliferation and evolution of metastatic lymphomas. Development of the syndrome was found to be regulated by the major histocompatibility complex with H-2 ^b mice being susceptible and H-2 ^d mice resistant. This model system may prove useful for understanding the pathogenesis of <u>HIV</u> infections in man.		

Project Number Z01 AI 00465-03 LIP

Other Investigators:

R. M. L. Buller	Senior Staff Fellow	LVD, NIAID
T. N. Fredrickson	Research Microbiologist	LIP, NIAID
D. M. Klinman	Investigator	IDB, NINCDS
M. Makino	Visiting Fellow	LIP, NIAID

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00484-02 LIP
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mechanisms in Hematopoietic Cell Differentiation		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI : K.L. Holmes Hd, Flow Cytometry Section BRB, NIAID Others: H.C. Morse, III Chief LIP, NIAID J.W. Hartley Hd, Viral Oncology Section LIP, NIAID R.A. Yetter Guest Researcher LIP, NIAID M.A. Principato Guest Researcher LIP, NIAID J.R. Hiernaux Visiting Fellow LI, NIAID P.J. Baker Senior Investigator LI, NIAID T.N. Fredrickson Guest Researcher LIP, NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Immunopathology		
SECTION Virology and Cellular Immunology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 5.0	PROFESSIONAL: 3.0	OTHER: 2.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) Initial studies of a wide variety of retrovirus induced tumors suggesting a close ontogenic relationship between the B cell and myeloid lineages have been complimented with experiments involving transfer of normal cells into immunodeficient or irradiated mice. We have found that, contrary to current views of hematopoietic cell development, the B cell and the myeloid lineages may share a common progenitor cell and that this cell may be identified by the expression of the putatively monocyte/macrophage restricted antigen Mac-1. Mac-1 was also found to be coexpressed with Thy-1 on a pluripotent stem cell. We have studied an antigen recognized by our new monoclonal antibody, LIP-6 which is expressed by Ly-1 B cells in the peritoneum and on peritoneal macrophages but not on peripheral B cells, T cells or granulocytes. This antigen may help to further elucidate the the relationship between the B and myeloid lineages. The effects of the BM-5 virus on the bone marrow was found to be most profound on B lymphopoeisis, initially stimulating production of B cells followed by a decrease in the ability of stromal cells to support the growth of normal cells. Studies of immunodeficient RIIIS/J mice have suggested, contrary to other reports, that within the Ly-1 B cell lineage, the related Ly-1⁺ and the Ly-1⁻ B cells may differ functionally.		

LABORATORY OF IMMUNOREGULATION
1988 Annual Report
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Summary Report
Laboratory of Immunoregulation
October 1, 1987 through September 30, 1988

Anthony S. Fauci, M.D.
Chief, Laboratory of Immunoregulation

Studies of the Human B Cell Cycle: Analysis of B Lymphocyte Activation Via Surface Receptors

Human B cells can be activated by antigen, anti- μ (which crosslinks surface Ig), Staphylococcus aureus Cowan I (SAC) (a mixture of bacterial cell wall proteins), or soluble factors produced by accessory cells. We have discovered a soluble factor produced by a helper T cell line which can directly activate resting B cells. This factor, called B cell activating factor (BCAF), may play a role in polyclonal B cell activation which occurs when T cells become stimulated (such as in various autoimmune diseases, acquired immunodeficiency syndrome [AIDS], etc.). We have been developing a strategy to purify enough of this protein to determine its amino acid sequence while we have also been trying to clone the RNA responsible for its production by using a frog oocyte injection system. At the same time, we have been examining some of the intracellular events which follow the binding of anti- μ or SAC to the B cell surface. Both anti- μ and SAC cause the generation of inositol phosphate and an intracellular calcium flux while only anti- μ induces the production of cAMP. At the same time SAC upregulates while anti- μ downregulates the expression of genes for Ig production. Subsequent studies revealed that any signal inducing cAMP will downregulate the expression of Ig genes. Thus, the mechanism of activating the B cell can determine which possible steps it can subsequently take. Our plans are to further define the intracellular events between activation and Ig gene expression, to clone BCAF and examine its relevance in normal and abnormal B cell physiology, and to define which of the proteins in SAC are responsible for its actions. (Young, Ambrus, Fauci, LIR/NIAID)

Studies of the Human B Cell Cycle: B Cell Specific Growth Factors

Our work in this area began 4 years ago with our discovery of a high molecular weight B cell growth factor (HMW-BCGF) produced by both B and T lymphocytes as well as the receptor to which it binds on activated B lymphocytes. Several other molecules have been described which enhance B cell proliferation including low molecular weight (LMW) BCGF which was described in this laboratory. We have found that these two BCGFs act on different but overlapping populations of B cells. This was shown not only in normal cells but also in various B cell tumors and immunodeficiency diseases. Patients with chronic lymphocytic leukemia and hairy cell leukemia respond primarily to HMW-BCGF, making the use of HMW-BCGF toxin conjugates or anti-HMW-BCGF toxin conjugates potential therapeutic modalities for these diseases. Patients with common variable immunodeficiency (CVI) respond primarily to LMW-BCGF and yet have receptors for HMW-BCGF. This brings up two interesting points: HMW-BCGF may be a necessary signal for a B cell function which these patients are missing and a post-receptor signaling defect might exist in the HMW-BCGF receptor of their B cells. Both of these points will be areas of future study. To perform the HMW-BCGF work more precisely and to allow examination of the genetic events involved with the use of HMW-BCGF, we are attempting to clone the genes both for HMW-BCGF and its receptor. This work has been showing slow but steady progress and will hopefully be finished in the next year. At the same time, we have been examining the intracellular events involved with BCGF induced proliferation. We have found that as in the activation stage, inositol phosphates and calcium are generated in response to BCGFs. Also, cAMP is generated but only in response to HMW-BCGF. Since cAMP is a negative signal for differentiation, this suggests that HMW-BCGF may have a role in

expanding memory B lymphocytes while LMW-BCGF may be expanding the B cell subpopulation which will progress to become plasma cells. Finally, we have demonstrated the phosphorylation of membrane protein following the binding of HMW-BCGF to its cellular binding site. We plan to expand our examination of all the intracellular signals utilized in human B cell proliferation including protein phosphorylations and the expression of various relevant genes. At the same time we will study the same intracellular events in B cell tumors, autoimmune diseases, and primary B cell immunodeficiency diseases.

Studies of the Human B Cell Cycle: B Cell Growth Factors that Are Not Entirely B Cell Specific

We have continued our studies on the role of cytokines which regulate B cell function, but which are not specific for B cells. The monocyte product, tumor necrosis factor- α (TNF- α), has BCGF activity and augments the differentiative effects of interleukin-2 (IL-2). Lymphotoxin, a related protein, has similar functional effects on human B cell function. Receptor crosslinking studies with iodinated TNF- α reveals a predominant species of 91 kD and identified 2 less prominent species of 74 and 55 kD. This suggests that the TNF- α receptor on human B cells consists of a multichain complex. B cell function is markedly suppressed by transforming growth factor-beta (TGF- β). Evidence supporting a role for TGF- β 1 as an autocrine downregulator of B cell function was found by using a neutralizing antibody to TGF- β . The related protein, TGF- β 2, has similar suppressive effects on B cell function as does TGF- β 1. Using chemical crosslinking, the TGF- β 1 receptor on normal B lymphocytes appears to be composed of 2 chains of 52 and 77 kD while only a single chain of approximately 50 kD is identified on transformed B cell lines. Additionally, binding experiments using iodinated TGF- β 1 and competitive inhibition with either TGF- β 1 or TGF- β 2 revealed marked differences between the normal B cells and the transformed cells. Thus, the observation that certain transformed B cell lines are refractory to the inhibitory effects of TGF- β may be accounted for by differences in their TGF- β receptor. Northern blots were performed to examine B cells for the expression of thyroid stimulating hormone receptor (TSH-R) mRNA. While unactivated B cells contained low levels of TSH-R mRNA, activated B cells were found to have significant quantities. Binding studies with iodinated TSH are in progress to confirm this observation. The addition of TSH to B cell cultures suppresses B cell function at high concentrations (1 nM) and augments B cell Ig production at low concentrations (10 pM). These results, in conjunction with previous data suggesting that lymphocytes secrete TSH, implicate TSH as a regulator of B cell function. Furthermore, the presence of POMC mRNA in human B cells and B cell lines has been shown. This observation provides further evidence in support of a role for neuro-endocrine hormones in the regulation of immune function. (Kehrl, Fauci, LIR/NIAID; Kohn, Oates, NIDDK).

Studies of the Human B Cell Cycle: The Differentiation of Human B Cells

We have continued our studies on the factors which are responsible for the induction of differentiation of human B cells. Two years ago we described a B cell differentiation factor (BCDF) secreted by a cloned T cell line which induces Ig secretion by normal B cells which have already gone into the proliferative cycle. Over the past year we have been purifying this factor for sequencing and cloning. We have identified the cellular receptor for this factor and are currently attempting to clone its gene. When BCDF binds its receptor, cGMP but not cAMP, calcium or inositol phosphates are generated. As noted above, cAMP will inhibit this stage of B cell function. Two other signals, prostaglandin E_2 (PGE $_2$) and LTC $_4$ were also found to inhibit Ig secretion. PGE $_2$ may act through stimulating adenylate cyclase and increasing cAMP while no clear mechanism for the action of LTC $_4$ has been found. Much more remains to be done in examining the events involved with both positive (BCDF) and negative (PGE $_2$, LTC $_4$) signals for differentiation. This work can all be applied then to examination of B cell differentiation in

various B cell disorders including CVI and systemic lupus erythymatosus (SLE). (Ambrus, Fauci, LIR/NIAID; Goldstein, Albert Einstein)

Analysis of B Cell Specific Gene Products

Several projects have been initiated to identify proteins important in B cell function. A B cell cDNA library was constructed from mRNA derived from B cells activated in vitro for 2 days. A portion of the library was screened and re-screened with a B-T cDNA probe. One hundred fifty cDNAs have been isolated which react with the B-T cDNA probe and not with Ig probes or a T cell cDNA probe. To date ten of the cDNAs have been partially sequenced and we are in the process of analyzing the sequence data. Additionally, Northern blots are in process to examine tissue specificity and regulation of expression of the mRNAs. Monoclonal antibodies are being raised against B cell membrane proteins in order to identify and clone other important cDNAs. Mammalian cDNA expression libraries have been made from activated normal B cells and from 2 B cell lines and will be used once interesting monoclonal antibodies have been isolated. Finally, 2D gels have been used to quantitate the differences between normal B and T cell membrane proteins. Computerized mapping demonstrates a 5% difference between activated T and B cell membrane proteins. In contrast, nearly a 20% difference was observed when resting and activated B cells were compared. Identification of proteins preferentially expressed in B cells is likely to lead to important insights in the control mechanisms that are important in B cell function. (Kehrl, Fauci, LIR/NIAID)

The Induction of Gene Expression in the Activation of Immune Competent Cells

We have continued our studies on the elucidation of the molecular events during activation of lymphoid cells and in particular during activation of human peripheral blood T cells. In this regard, we have studied the primary transcriptional response of human peripheral blood T cells to mitogenic activation. Previously we had constructed a subtractive cDNA library and used subtractive and differential screening methods to isolate genes which are induced early on in T cells stimulated with mitogens. The limited number of known inducible genes encode oncogenes, lymphokines and their receptors, i.e., genes with significant roles in proliferation and/or initiation of an immune response. To date we have isolated in excess of 69 induced genes, which include several of the known genes but which represent a much larger number than could have been expected from previous studies. We are pursuing both functional and regulatory aspects of the many novel induced genes. By using mitogenic antibodies to the CD2 and CD3 receptors as well as the co-mitogens anti-CD28, phorbol myristate acetate (PMA), DIC8 and ionomycin, we were able to determine that every mitogenic agent induces all genes checked to date but that the co-mitogens could easily distinguish these genes into many different groups with common regulatory elements. Two genes, 464 and 744, behaved similar to IL-2. Approximately one fourth of the genes tested are not expressed in activated human lung fibroblasts, including 464 and 744. These genes are also among those which are very sensitive to the immunosuppressive drug cyclosporin A and among those which are constitutively expressed in human T lymphotropic virus I (HTLV-I) transformed cells. That these genes possibly encode lymphokines was further supported by their primary sequence, which predicts encoded proteins with typical N-terminal leader sequences and with homologies to each other as well as to a group of so-called inflammatory response genes, a new family of factors which is just emerging. In cell lines permanently transfected with the human immunodeficiency virus (HIV-1) orf-B protein the induction of some genes, including 464, appears to be negatively affected. In addition to these studies, we have discovered several DNA binding proteins which interact with the mitogen-responsive region of the IL-2 gene. Some of these proteins bind DNA only upon prior activation of the cells. Also, we have delimited further the segment of the c-myc gene, which controls the downregulation of this gene during terminal differentiation. Lastly, we have

continued to attempt indirect methods of cloning a BCGF and its receptor. (Siebenlist, Zipfel, Schmidt, Bressler, Brunvand, LIR/NIAID; Kelly, IB/NCI, Rapp, FCRF/NCI)

Immunopathogenic Mechanisms of HIV Infection

Over the past several years, the LIR has been actively studying the mechanism of immunopathogenesis of HIV infection. With the definitive identification of HIV as the causative agent of AIDS in 1984, we began a series of studies aimed at delineating these mechanisms at the cellular and molecular levels.

The mechanisms whereby a latent or low-level, chronic infection with HIV is converted to an active, productive infection have been of particular interest to the LIR. Over the past year we have intensified these efforts. We have developed chronically infected promonocyte and T cell lines which have been successfully cloned. These have served as *in vitro* models of chronically infected monocytes and T cells, a state which is generally believed to exist *in vivo*. The infected promonocyte clone (U1) does not constitutively secrete virus, but can be induced to express virus by a variety of stimuli such as PMA. The infected T cell clone (ACH-2) constitutively expresses virus in approximately 10 to 15% of cells and can be induced to express virus in virtually 100% of cells following induction with PMA. We employed these models to determine whether physiological factors in the form of cytokines which are normally secreted in response to immunologic stimuli, would be capable of inducing virus expression. We had previously demonstrated that mitogens, antigens, and transfected heterologous virus genes could induce virus expression. However, it was of obvious importance to determine if virus expression would be inexorably induced during normal immunologic responses.

We demonstrated that cytokine-rich supernatants from phytohemagglutinin (PHA)-stimulated mononuclear cells readily induced HIV expression from both the U1 and ACH-2 lines. We then demonstrated that monokines obtained from lipopolysaccharide-stimulated, highly purified monocytes also induced virus expression. In addition, recombinant granulocyte/macrophage colony stimulating factor (GM-CSF) induced HIV expression, but a variety of other recombinant cytokines such as IL-1, IL-2, IL-3, IL-4, and interferon (IFN)- γ did not. Of particular interest is the fact that TNF- α , known as cachectin, as well as TNF- β , known as lymphotoxin, were extremely potent in the induction of HIV from both cell lines. Given the fact that TNF- α is secreted in high amounts in a variety of infections as well as the fact that TNF- α levels are elevated in AIDS patients, the demonstration of the HIV-inductive capability of this factor has obvious immunopathogenic and clinical implications.

Using transient transfection experiments with the long terminal repeat (LTR) from HIV linked to the CAT gene (LTR-CAT), we demonstrated that inductive effects of these cytokines were due to a transactivating mechanism with the target being the LTR of HIV. This is compatible with the well-recognized mechanisms of induction of transcription since the start site of transcription of the HIV provirus resides on the LTR. Finally, we have demonstrated that the induction of transcription results from the binding of DNA-binding proteins to certain specific sites on the LTR.

Using these chronically infected cell lines, we further demonstrated that IFN- α , but not azidothymidine (AZT) was capable of blocking the induction of complete expression of HIV in an already infected cell. The mechanism of blocking was an interference with the late stage of virus expression, i.e., the budding of virions from the cell surface at the same time that virus proteins were synthesized in normal amounts. Since AZT could effectively block *de novo* acute infection of target cells, these experiments provide a firm scientific basis for the use of AZT in combination with IFN- α since it is likely that new acute infection of susceptible targets as well

as the induction of expression of HIV in already infected cells occurs continuously in HIV infected individuals.

We have continued our studies on the in vivo and in vitro infection of peripheral monocytes by HIV. We demonstrated that HIV infects monocytes in an indolent and chronic manner with very little cytopathic effect. Virus buds and accumulates intracellularly and is not readily released from infected cells. These observations are compatible with the concept that monocytes serve as a reservoir of HIV in the body and can likely disseminate the virus to various organs.

Using a novel positive selection technique, we were able to isolate highly purified progenitor cells from normal human bone marrow. We infected these cells with HIV and succeeded in demonstrating for the first time that myelomonocytic cells could be infected with HIV. The virus budded intracellularly and accumulated in copious amounts in these progenitor cells in the absence of significant cytopathic effects, a situation remarkably similar to that seen in mature monocytes and in contrast to that in CD4+ T lymphocytes. These observations may explain certain of the hematologic abnormalities seen in HIV infection. In addition, these findings indicate that the bone marrow may serve as a potentially important reservoir of HIV in the body.

We extended our studies aimed at determining the effects of HIV infection on the T cell pool. We demonstrated that HIV infection of highly purified CD4+ peripheral blood T cells resulted in the outgrowth of T cell lines which were CD4-/CD8- and expressed the gamma-delta T cell receptor. These cells appeared to be unable to generate the production of mRNA specific for IL-2 following stimulation with PHA and PMA. In a parallel series of studies it was found that there was an increase in the percentage and absolute count of these same gamma-delta cells in the peripheral blood of HIV-infected patients. (Folks, Koenig, Schnittman, Poli, Lane, Stanley, Vitkovic, Massari, Fauci, LIR/NIAID; Martin, Rabson, LMM/NIAID; Fox, NCI; Kessler, NMRI).

The Immune Response to HIV Infection

Over the last few years we have been studying the immune response to HIV infection. Over the past year we developed for the first time HIV-specific, MHC restricted T cell clones from the peripheral blood of HIV infected individuals. The availability of these T cell clones should allow us to delineate the fine specificity of the cytotoxic T cell response to HIV.

Over the past year we developed a novel assay to measure the presence and levels of antibodies which block the binding of the HIV envelope protein to the CD4 molecule on the susceptible target cell. Studies are underway to determine the clinical relevance if any of these antibodies. (Koenig, Fauci, LIR/NIAID; Moss, Earl, LVD/NIAID)

Clinical Investigation in AIDS

An ongoing study of 983 health care workers continues to demonstrate that the risk of HIV transmission from patient to health care worker is low. Despite 126 percutaneous exposures, 337 mucosal exposures, and 1672 cutaneous exposures to blood or body fluids from individuals with HIV infection, no evidence of seroconversion to HIV has been found.

In an effort to establish better diagnostic techniques for detection of HIV infection, 362 homosexual or bisexual men with a history of high-risk behavior and a negative ELISA test for antibody to HIV were examined by Western blot, p24 antigen capture, co-culture for HIV,

immunologic profile and in some instances polymerase chain reaction (PCR). Thirty percent of individuals were found to have one or more bands on Western blot. From a cohort of twenty of these individuals, three were positive by PCR.

A series of clinical trials were carried out in an effort to develop better therapies for the underlying HIV-1 infection and immunologic defects seen in patients with AIDS and related conditions. IFN- α was found not only to have anti-neoplastic activity but also anti-retroviral activity as well when administered to patients with early stages of HIV-1 infection. AZT was demonstrated to have some degree of benefit in patients with AIDS and Kaposi's sarcoma. A combination trial of these two agents in patients with AIDS-associated Kaposi's sarcoma appeared to yield better results than either agent alone and with less toxicity. Phase I studies with muramyl tripeptide have demonstrated the ability to enhance tumoricidal activity of peripheral blood monocytes from patients with AIDS. Bone marrow transplantation in association with adoptive transfer of syngeneic lymphocytes in patients pre-treated with AZT has failed to yield better results than that achieved with either modality alone.

The first U.S. trial of a candidate AIDS vaccine was initiated utilizing the full length envelope precursor protein of HIV-1, gp160. Individuals immunized with up to 160 μ g of gp160 have developed humoral and/or cell mediated immune response to immunogen, other recombinant envelope proteins and native envelope protein. (Lane, Fauci, Davey, Deyton, LIR/NIAID; Masur, Kovacs, Falloon, Polis, Henderson, CC/NIH)

International Studies on HIV Infection

AIDS has become a global pandemic, with cases reported in 138 countries throughout the world. An intensive research effort has been undertaken in several countries in the Caribbean and Africa to study the unique epidemiologic, virologic, clinical, and immunologic features of AIDS in these areas. In Kinshasa, Zaire, we identified over 10,000 cases of AIDS in 1987 with a male to female ratio of 1:1.5. The disease is predominantly heterosexually transmitted with high seroprevalence rates to the HIV of 3% to 6% among the general heterosexual population in Kinshasa in contrast to <1% in rural Africa. HIV infection rates of 20% to 80% have been documented among spouses of AIDS patients, female prostitutes, and among patients attending sexually transmitted disease (STD) clinics. Similar studies in the United States have confirmed a 5% infection rate among patients attending STD clinics with increasing evidence of heterosexual transmission among these patients. The presence of genital ulcerations were significantly associated with heterosexual transmission. In studies of perinatal transmission of HIV infection, over 15,000 pregnant women in Haiti, Kenya, and Zaire, of whom approximately 6% are HIV seropositive, have been enrolled in prospective studies. Perinatal transmission which occurs in approximately 50% of children appears to correlate with advanced clinical disease and immunologic impairment of the mother with depressed T4 cells. Perinatal HIV infection was associated with premature birth, low birth weight, and an increased mortality (22%) within the first year of life. Childhood immunization studies on HIV-infected children demonstrated efficacy of most vaccines with few adverse effects, documenting the continual need for complete immunization programs. Immunologic studies have confirmed the development of HIV-1 specific antibody dependent cellular cytotoxicity in HIV-infected African patients which correlates strongly with the development of antibody to gp41. Further studies will continue to examine the frequency of human retroviral infection (HIV-1, HIV-2, HTLV-1) in selected populations with particular emphasis on natural history, perinatal transmission, and immunopathogenesis which might have relevance to future vaccine studies. (Quinn, Brown, Francis, Fauci, LIR/NIAID; Ryder, CDC; Piot, Colebunders, Institute of Tropical Medicine, Antwerp, Belgium; Holmes, Kreiss, U. of Washington; Hook, Halsey, Johns Hopkins)

Clinical, Immunopathogenic, and Therapeutic Studies in the Spectrum of Vasculitis

The LIR is currently studying prospectively one of the largest groups of patients with the vasculitic syndromes in the world. On the basis of clinical, pathophysiologic, immunopathogenic, and therapeutic results obtained over the last 20 years, we have designed a revised categorization scheme for the vasculitides which has now reached worldwide acceptance. On the basis of our observations, lymphomatoid granulomatosis is classified as a subset of malignant lymphoma and not as a primary vasculitis. We have described a new vasculitic syndrome termed the polyangiitis overlap syndrome. We have developed and instituted aggressive chemotherapeutic regimens consisting of chronically administered cyclophosphamide together with corticosteroids. This therapy has dramatically altered the 5-year survival in patients with systemic vasculitis from 13% in untreated patients to 48% after treatment with corticosteroids to greater than 90% in our patients treated with both cyclophosphamide and corticosteroids. This therapeutic protocol was originally evaluated in our patients with Wegener's granulomatosis, which now number over 150, but has been extrapolated and successfully used in treating other vasculitides such as polyarteritis nodosa, allergic angiitis and granulomatosis, isolated central nervous system vasculitis, Takayasu's arteritis, the acute vasculitis of Sjögren's syndrome, and the polyangiitis overlap syndrome. Our therapeutic protocols for the vasculitic syndromes are now used worldwide. We have observed certain unexpected long-term side effects of cyclophosphamide and are currently developing new therapeutic protocols for the vasculitic syndromes. In this regard, we have begun to treat patients who cannot tolerate or fail to respond to cyclophosphamide therapy with cyclosporin A. We have also begun to study the efficacy and toxicity of monthly pulse cyclophosphamide in the vasculitic syndromes. Finally, we have been liberally employing surgical correction of stenotic lesions in patients with Takayasu's arteritis resulting in remarkably favorable functional improvement and survival rates. (Fauci, Leavitt, Hoffman, LIR/NIAID; Parrillo, Shelhamer, CC/NIH; Giordano, George Washington U. Medical Center)

Studies of the Immunopathogenic Features of Immune-Mediated Diseases

The immunopathogenic mechanisms of a number of immune-mediated diseases and/or diseases characterized by abnormalities of immune function were investigated. B cells from patients with CVI were studied and shown to respond normally to LMW-BCGF, but abnormally or not at all to HMW-BCGF despite the ability to express receptors for HMW-BCGF. This abnormality is compatible with a post receptor signal transduction defect. Indeed, our data suggest that overproduction of an arachidonic acid metabolite such as PGE or one of the leukotrienes may be playing an inhibitory role in the normal signal transduction occurring with HMW-BCGF. Use of the lipoxigenase inhibitors ketoprofen or hydrocortisone have resulted in markedly enhanced production of antigen-specific antibody in vitro. We have extended our studies on the hypereosinophilic syndrome and have recently had the opportunity to investigate a large family cohort of patients with hereditary hypereosinophilia. They will serve as a valuable model for study of this interesting syndrome. We have demonstrated the presence of antineutrophil antibodies in patients with Wegener's granulomatosis, lymphomatoid granulomatosis, polyarteritis nodosa, and SLE. In some cases, the titer of antineutrophil IgG correlates with disease activity. Finally, we have continued our studies of idiopathic dilated cardiomyopathy and preliminary analysis of data indicate that this disease is responsive to immunosuppressive therapy. (Fauci, Ambrus, Leavitt, Hoffman, Young, LIR/NIAID)

Immunopathogenesis of Chlamydia Trachomatis

Chlamydia trachomatis is the most common sexually transmitted bacterial pathogen in the United States with an estimated 20 million cases annually. Studies have been in progress to define the clinical spectrum of chlamydial infection, to develop rapid diagnostic assays, and to examine the pathogenesis of chlamydial infections in experimental animal models. In women, chlamydia cervical infection continues to be high (20% to 30%). Coinfection with gonorrhea and human papillomavirus frequently occur and are strongly associated with the appearance of cervical dysplasia and cervical carcinoma. In a study to determine the reliability of mucopurulent cervicitis (MPC) as a predictive sign for chlamydial infection, we found that 51% of patients with MPC had gonococcal cervicitis and 44% had chlamydial cervicitis, while 28% had both. Contrary to previous studies, no significant association between chlamydia and polymorphonuclear leukocytes or with MPC could be established. The positive predictive value of MPC for chlamydial infection was only 44% due to the frequent occurrence of other infections such as gonorrhea and trichomonas. Studies have been initiated to examine the impact of contraceptives on the recurrence of chlamydial infections. In a population with a chlamydial infection rate of 26% at time of enrollment, only 9% demonstrated infection following active use of contraceptives. Diagnostic assays including in situ hybridization utilizing DNA and RNA probes for *C. trachomatis* as well as polymerase chain-reaction have been initiated, and a high sensitivity and specificity for detection of chlamydia in tissue samples has been documented. A series of investigations concerning biology and pathogenesis of the TWAR strain of chlamydia are currently in progress. We have demonstrated that optimal growth of TWAR is achieved in HeLa cells following 120 hour incubation, and that TWAR can be detected with monoclonal antibodies directed at the lipopolysaccharide antigen of chlamydia. Inoculation of cynomolgus monkeys with TWAR demonstrated establishment of mucosal infections of the eye, nasopharynx, and intestine, followed by a serologic immune response to the infecting agent. Further studies are planned to examine the clinical spectrum of chlamydial infections and the immunopathogenesis of this pathogen in non-human primate models. (Quinn, LIR/NIAD)

Future Plans and Objectives

Over the past year, the LIR has made a number of significant contributions in the areas of the regulation of the human immune response in normal and disease states, the immunopathogenic mechanisms of infection with the human immunodeficiency virus (HIV), clinical studies in AIDS, and studies of the immunopathogenesis of immune-mediated diseases.

We will continue our studies on the activation pathways which are triggered following stimulation of resting B cells with factors such as B cell activation factor (BCAF) as well as anti- μ and Staphylococcus aureus Cowan I. We will attempt to clone the genes for BCAF and its cellular receptor.

We will continue in our attempts to clone the genes for the high molecular weight B cell growth factor (BCGF) and its receptor. We will also extend studies on the second messenger pathways of B cell activation following triggering with the B cell specific factors.

We will continue our studies on immune-mediated diseases that manifest abnormalities of function BCGF receptors.

We will continue our attempts to clone the genes for B cell differentiation factor and its receptor as well as further delineate functional correlates of cell--factor interactions.

We will continue to analyze B cell specific gene products to get further insight into the molecular mechanisms of B cell function.

We will extend our studies on the induction of gene expression associated with the activation of immune competent cells. We have already isolated several novel T cell specific genes. We will attempt to isolate and characterize their gene products and continue to pursue the study of other novel genes.

We will pursue the delineation of the mechanisms whereby a latent or low-level, chronic infection with HIV is converted to an active, productive infection. This area will be pursued with full emphasis on the molecular biological approach.

We will pursue studies on the infection of normal bone marrow precursors with HIV and extend these studies to patients already infected with the virus. In addition, we will attempt to infect thymic precursors with HIV.

We will pursue our studies of HIV infection of monocytes in an attempt to delineate the molecular mechanisms of intracellular propagation of virus in monocytes.

We will utilize our development of HIV-specific, MHC-restricted T cell clones to study the fine specificity of the cytotoxic T cell response to HIV.

. We will continue our phase I vaccine trial of the gp160 HIV vaccine candidate.

We will continue and extend our clinical trials program of anti-HIV drugs and immunomodulators.

We will continue our study of syngeneic bone marrow transplantation in HIV infection with the added possibility of immunizing the donors with HIV proteins in accordance with our vaccine study in order to transfer immunity against HIV.

We will continue our epidemiologic studies of HIV-infected individuals.

The longstanding studies of the use of cytotoxic agents together with alternate-day corticosteroids in systemic vasculitis have continued with full confirmation of the original dramatically favorable results. In addition, we have instituted alternate protocols of cyclosporin A or bolus cyclophosphamide in individuals who cannot tolerate chronic low-dose cytotoxic therapy.

Honors, Awards, and Scientific Recognition

Over the past year, members of the LIR have received a number of awards and honors.

In 1988 Dr. Fauci was appointed Director, Office of AIDS Research, NIH, and Associate Director of NIH for AIDS Research. He continues to serve on a number of committees of scientific and administrative importance. During the past year he served on the International Advisory Committee for the IVth International Conference on AIDS in Stockholm and was appointed to the NIH Resource Allocation Group.

Dr. Quinn continues to serve on several advisory boards on AIDS and on sexually transmitted diseases, including the Advisory Committees on AIDS to the Institute of Medicine of the National Academy of Sciences and to the Pan American Health Organization. He also is on the NIAID Advisory Committee on Sexually Transmitted Diseases and the Scientific Advisory Board to the Caribbean Epidemiology Center in Trinidad. In addition, Dr. Quinn served on the scientific committee for the IVth International Conference on AIDS.

Dr. Fauci and other members of the LIR serve on editorial boards of journals concerned with the areas of immunology, allergy, and infectious diseases. Dr. Fauci continues his role as Associate Editor for the American Journal of Medicine. He maintains his position on the editorial boards of Annals of Allergy, Clinics in Immunology and Allergy, La Ricerca in Clinica e in Laboratorio, Physicians' Journal Update, Journal of Immunopharmacology, Cellular Immunology, EOS, Immunopharmacology, The Journal of Molecular and Cellular Immunology, and AIDS Research and Human Retroviruses. In addition, he continues his appointment as an advisory editor for North America for the journal Thymus and as advisory editor for The Journal of Clinical Immunology. During the past year he was appointed to the advisory board of Clinical Immunology and Immunopathology and to the editorial board of AIDS Patient Care.

During the past year, Dr. Quinn became a member of the editorial board of AIDS, AIDS Research and Human Retroviruses, The Journal of the Acquired Immunodeficiency Syndrome, and AIDS Clinical Digest. Dr. Lane joined the editorial boards of The Journal of Clinical Immunology, Clinical Immunology and Immunopathology, AIDS and Human Retroviruses, and The Journal of the Acquired Immunodeficiency Syndrome.

Dr. Fauci continues to edit with Dr. John I. Gallin the book series "Advances in Host Defense Mechanisms." In addition, he is an editor of Harrison's Principles of Internal Medicine" and "Harrison's Principles of Internal Medicine - Companion Handbook." He also co-edits the textbooks "Current Therapy in Allergy, Immunology and Rheumatology-3" and "Current Therapy in Internal Medicine." Dr. Quinn is co-editor of "Topics in the Acquired Immunodeficiency Syndrome," Volume II. Finally, Dr. Fauci and others have contributed a number of invited chapters covering a variety of subjects to most of the major textbooks of medicine as well as subspecialty textbooks in immunology, allergy, and infectious diseases.

Dr. Fauci as well as several members of the LIR were asked to deliver major named or invited lectureships during the year. Dr. Fauci was the Distinguished Lecturer in Medical Science at the Mayo Graduate School of Medicine, and he delivered the University Lectureship at the University of Texas Health Science Center at Dallas. Dr. Fauci was an invited symposium speaker at the 4th International ARC/NIH Symposium - Cancers in the Developed World. He was an invited plenary speaker at the 27th Annual Meeting of the International Conference on Antimicrobial Agents and Chemotherapy, the Annual Meeting of the Hospice Association of America, The American College of Physicians District of Columbia Annual Meeting, the Annual

Meeting of the National Academy of Sciences, and the Centennial Symposium of Abbott Laboratories. He was also an invited plenary lecturer at the International Symposium on Immunoregulation in Clinical Diseases in Florence, Italy, and at the annual meetings of the American Academy of Pediatrics and of the American Association of Immunologists. Dr. Fauci also delivered the Alpha Omega Alpha Lecture to the Uniformed Services University of the Health Sciences and to the New York University School of Medicine. Of particular note was the Kober Lecture which he delivered to the Association of American Physicians.

During the past year, Dr. Koenig was an invited lecturer at several national and international meetings: International Symposium on Infectious Diseases in Tokyo, the First International Conference on the GI Tract and the Liver in the Acquired Immunodeficiency Syndrome in Munich, and the Brazilian Society of Immunology in Sao Paulo, Brazil, as well as the American Academy of Dermatology Annual Meeting, the American Medical Association Conference: Biotechnology Targets AIDS: A Clinical Perspective and An International Symposium: Viruses, Rickettsiae, and Severe Human Disease: Natural History and Prevention.

Dr. Lane delivered the 5th Annual Julius Stone Lecture of the American Dermatologic Society for Allergy and Immunology. Dr. Ambrus was an invited lecturer at the Leukocyte Culture Conference and the International Society for Experimental Haematology. Dr. Siebenlist delivered invited lectures at the B Cell Neoplasia Meeting and the Gordon Conference on Animal Cells and Viruses and Mitogen Induced Genes in Human T Cells.

Finally over the past year, Dr. Fauci was honored by receiving the Public Health Leader of the Year Award of the Commissioned Officers Association of the U.S. Public Health Service, the Kober Lecture Award of the Association of American Physicians, and the National Institutes of Health Clinical Center Annual Distinguished Clinical Educator Award. In addition, Dr. Fauci's work was cited in the 1988 Citation Classics (Current Contents) for the article - Fauci AS, Haynes BF, Katz P: The spectrum of vasculitis. Ann Intern Med 89: 660-676, 1978.

Administrative, Organization, and Other Changes

Established in 1980, the Laboratory of Immunoregulation (LIR) is in its eighth year. The major theme of the laboratory continues to be the delineation of the mechanisms of regulation of the human immune response in normal and disease states. Over the past year, greater emphasis and resources have been placed on the study of the human immunodeficiency virus (HIV) and AIDS. Dr. Fauci was appointed as the Director of the newly created Office of AIDS Research of NIH as well as the Associate Director of NIH for AIDS Research. The AIDS Clinical Studies Group under the leadership of Dr. H. Clifford Lane has been expanded over the past year and this group will become an official section within the LIR within the next year. The group now consists of Drs. Lane, Joseph Kovacs, Richard Davey, and Judith Falloon. In addition, several study nurses have been added to the team.

Intensive activity continues in the area of immunopathogenic mechanisms of HIV infection. This year, Dr. Ljubisa Vitkovic has joined the group as a senior staff fellow on a tenure track. Dr. Vitkovic will be studying pathogenic mechanisms of the neurologic manifestations of HIV at the molecular level. Dr. Thomas Folks will be leaving the LIR in the fall of 1988 to assume a position as Laboratory Chief at the Centers for Disease Control in Atlanta. Drs. Scott Koenig and Peter Bressler have entered their fifth year as of July 1988 as senior staff fellows. Dr. Bressler completed his training in molecular biology under Dr. Ulrich Siebenlist of LIR and will concentrate on the molecular biology of HIV. Dr. Steven Schnittman has entered his fourth year as a medical staff fellow, and Drs. Ferdinand Massari and Guido Poli will continue on for an additional year. Dr. K. Randall Young has completed his fellowship and has joined the faculty at the University of Alabama in Birmingham. Drs. Randi Leavitt and Gary Hoffman continue their responsibility of assisting Dr. Fauci in the non-AIDS clinical research studies of LIR. Drs. Julian Ambrus and John Kehrl continue on as senior investigators studying the regulation of the human B cell cycle. Drs. Peter Zipfel and Albrecht Schmidt continue on for an additional year in Dr. Siebenlist's group, while Dr. Mark Brunvand has finished his fellowship with Dr. Siebenlist and has joined the faculty at the University of Washington.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00210-08 LIR

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunoregulation of Human Lymphocyte Function in Normal and Disease States

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	A.S. Fauci	Chief	LIR, NIAID
Others:	J.H. Kehrl	Senior Investigator	LIR, NIAID
	J.L. Ambrus, Jr.	Senior Investigator	LIR, NIAID
	A. Taylor	Staff Fellow	LIR, NIAID
	U. Siebenlist	Senior Staff Fellow	LIR, NIAID
	K.R. Young	Medical Staff Fellow	LIR, NIAID
	P. Bressler	Medical Staff Fellow	LIR, NIAID

COOPERATING UNITS (if any)

BRB, NIAID (J. Coligan); LI, NIAID (T. Chused); NEI (M. Roy); Washington Univ. (E.J. Brown, M. Peters); Duke Univ. (W.C. Greene, M. Dukovich, P. Buckley); American Red Cross (A. Tenner); NCI (S. Rudikoff, A. Roberts, M. Sporn, Lê thi Bich-Thuy); Madrid, Spain (M. Alvarez-Mon); Univ.

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

7

PROFESSIONAL:

4

OTHER:

3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

We have continued our studies on the mechanisms of activation, proliferation, and differentiation of human lymphocytes, particularly B cells, at the cellular and molecular levels. By examining intracellular, second messenger events following triggering of B cells with anti- μ , Staphylococcus aureus Cowan I (SAC), or a B cell activating factor (BCAF), we have demonstrated divergent pathways of activation associated with expression of different cellular function. We have extended our studies on the B cell specific growth factors (BCGF): high molecular weight (HMW) and low molecular weight BCGFs. We have demonstrated that these factors expand different functional subsets of B cells. In addition, we have shown that B cells from patients with diseases such as common variable hypogammaglobulinemia (CVH) or a variety of B cell leukemias express receptors to these factors to variable levels and are responsive to the factors in a manner discordant with the expression of receptors for the factors. We have delineated the intracellular signals involved in B cell proliferation to our BCGFs and have demonstrated that the receptor for HMW-BCGF is phosphorylated upon binding of factor to its cellular binding site. Attempts at cloning the genes for the HMW-BCGF and its receptor continue. In parallel, we have continued studies on BCGFs that are potent inducers of B cell function, but which are not entirely B cell specific. We have demonstrated that transforming growth factor- β (TGF- β) is an inhibitory autocrine lymphokine that may play a role in physiologic downregulation of B cell function. In addition, we have characterized the TGF- β receptor on human B cells. We have demonstrated that lymphotoxin and tumor necrosis factor- α (TNF- α) are important B cell regulatory factors and have shown the upregulation of TNF- α receptors following B cell activation. Additional studies suggest that the TNF- α receptor on human B cells is a multichain complex. We have recently initiated studies aimed at analyzing B cell specific gene products. A cDNA library has been constructed from activated B cell mRNA and screened with B minus T cell mRNA probes.

Others:	T. Nakagawa	Visiting Fellow	LIR, NIAID
	N. Nakagawa	Visiting Fellow	LIR, NIAID

Cooperating Units:

Chicago (M. Gurney); Univ Minnesota, Minneapolis (F. Uckun); Amgen Corp, Thousand Oaks, CA (E. Medlock, K. Rosley, S. Suggs, M. Johnson, T. Lu, L. Soussa); Harvard Medical School and Syntex Corp. (R.A. Lewis); Einstein Medical College, Bronx, NY (H. Goldstein, N. Salvatore); WRAMC, Washington, DC (R. Engler); University Hospital Groningen, The Netherlands (F.J. van der Woude).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00212-08 LIR

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of the Immunopathogenic Features of Immune-Mediated Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	A.S. Fauci	Chief	LIR, NIAID
Others:	J.L. Ambrus, Jr.	Senior Investigator	LIR, NIAID
	R.Y. Leavitt	Senior Investigator	LIR, NIAID
	K.R. Young	Medical Staff Fellow	LIR, NIAID
	G.S. Hoffman	Expert	LIR, NIAID

COOPERATING UNITS (if any)

CC, NIH, (J.E. Parrillo); Mayo Clinic, Rochester, MN, (G. Gleich); Univ. Minnesota, (F. Uckun); Duke Univ., (R. Buckley); Georgetown Univ. (P. Katz, T. Cupps); SUNY, Buffalo, (D. Farolino, G. Logue); Speciality Laboratories, (J. Peter, S. Wormsley); LPD, NIAID (D. Kaslow).

LAB BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

1

PROFESSIONAL

1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects	☐ (b) Human tissues	☐ (c) Neither
☐ (a1) Minors		
☐ (a2) Interviews		

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The immunopathogenic mechanisms of a number of immune mediated diseases and/or diseases characterized by abnormalities of immune function were investigated. B cells from patients with common variable immunodeficiency (CVI) were studied and shown to respond normally to low molecular weight B cell growth factor (LMW-BCGF), but abnormally or not at all to high molecular weight (HMW)-BCGF despite the ability to express receptors for HMW-BCGF. This abnormality is compatible with a post receptor signal transduction defect. Indeed, our data suggest that overproduction of an arachidonic acid metabolite such as PGE or one of the leukotrienes may be playing an inhibitory role in the normal signal transduction occurring with HMW-BCGF. Use of the lipoxigenase inhibitors ketoprofen or hydrocortisone have resulted in markedly enhanced production of antigen specific antibody in vitro. We have extended our studies on the hypereosinophilic syndrome (HES) and have recently had the opportunity to investigate a large family cohort of patients with hereditary hypereosinophilia. They will serve as a valuable model for study of this interesting syndrome. We have demonstrated the presence of antineutrophil antibodies in patients with Wegener's granulomatosis, lymphomatoid granulomatosis, polyarteritis nodosa, and SLE. In some cases, the titer of anti-neutrophil IgG correlates with disease activity. Finally, we have continued our studies of idiopathic dilated cardiomyopathy and preliminary analysis of data indicate that this disease is responsive to immunosuppressive therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00213-08 LIR
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Clinical, Immunopathogenic, and Therapeutic Studies in the Spectrum of Vasculitis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: A.S. Fauci Chief LIR, NIAID Others: R.Y. Leavitt Senior Investigator LIR, NIAID G.S. Hoffman Expert LIR, NIAID		
COOPERATING UNITS (if any) Critical Care Medicine Department, Clinical Center, NIH (J.E. Parrillo, J. Shelhamer); George Washington University Medical Center (J. Giordano).		
LAB/BRANCH Laboratory of Immunoregulation		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1	PROFESSIONAL: 1	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The LIR is currently studying prospectively one of the largest groups of patients with the <u>vasculitic syndromes</u> in the world. On the basis of clinical, pathophysiologic, immunopathogenic, and therapeutic results obtained over the last 20 years, we have designed a revised <u>categorization scheme</u> for the vasculitides which has now reached worldwide acceptance. On the basis of our observations, <u>lymphomatoid granulomatosis</u> is classified as a subset of malignant lymphoma and not as a primary vasculitis. We have described a new vasculitic syndrome termed the <u>polyangiitis overlap syndrome</u>. We have developed and instituted aggressive chemotherapeutic regimens consisting of chronically administered <u>cyclophosphamide</u> together with <u>corticosteroids</u>. This therapy has dramatically altered the 5-yr <u>survival</u> in patients with systemic vasculitis from 13% in untreated patients to 48% after treatment with corticosteroids to <u>greater than 90%</u> in our patients treated with both cyclophosphamide and corticosteroids. This therapeutic protocol was originally evaluated in our patients with <u>Wegener's granulomatosis</u> which now number over 150, but has been extrapolated and successfully used in treating other vasculitides such as <u>polyarteritis nodosa</u>, <u>allergic angiitis and granulomatosis</u>, <u>isolated central nervous system vasculitis</u>, <u>Takayasu's arteritis</u>, the <u>acute vasculitis of Sjogren's syndrome</u>, and the <u>polyangiitis overlap syndrome</u>. Our therapeutic protocols for the vasculitic syndromes are now used worldwide. We have observed certain unexpected long-term side effects of cyclophosphamide and are currently developing new therapeutic protocols for the vasculitic syndromes. In this regard, we have begun to treat patients who cannot tolerate or fail to respond to cyclophosphamide therapy with cyclosporin A. We have also begun to study the efficacy and toxicity of monthly pulse cyclophosphamide in the vasculitic syndromes. Finally, we have been liberally employing surgical correction of stenotic lesions in patients with Takayasu's arteritis resulting in remarkably favorable functional improvement and survival rates.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 A1 00390-05 LIR

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Investigation of the Acquired Immunodeficiency syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H.C. Lane	Senior Investigator	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	R.T. Davey	Medical Officer	LIR, NIAID
	M. Easter	Nurse Practitioner	LIR, NIAID
	D. Lee	Project Coordinator	LIR, NIAID
	V. Davey	Project Coordinator	LIR, NIAID
	L. Deyton	Medical Staff Fellow	LIR, NIAID

COOPERATING UNITS (if any)

CC, NIH (H. Masur, J. Kovacs, J. Falloon, M. Polis, D. Henderson)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

8

PROFESSIONAL

2

OTHER

6

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF NCRP (Use standard unreduced type. Do not exceed the space provided.)

An intensive effort was directed toward studying the epidemiologic, virologic, immunologic and therapeutic aspects of the acquired immunodeficiency syndrome. An ongoing study of 983 health care providers has continued to demonstrate the low risk of HIV-1 transmission from patient to hospital worker. The polymerase chain reaction was used to identify the presence of HIV-1 genome in 3 out of 20 individuals with negative ELISA tests and indeterminate antibody profiles on Western blot. HIV-1 infection of highly purified peripheral blood CD4+ T cells has been shown to result in the outgrowth of a population of gamma-delta T cells that are defective in IL-2 production. A subset of patients with HIV-1 infection were shown to have increased numbers of gamma-delta cells in the peripheral blood. Immunization of healthy human volunteers to the gp160 envelope precursor protein of HIV-1 has elicited antibody and lymphocyte blast transformation responses to both recombinant and natural product HIV-1 proteins. A series of clinical trials were carried examining the effects of alpha interferon and AZT either alone or in combination in patients with early stages of HIV-1 infection. Attempts at immunologic reconstitution employed the immunomodulators IL-2 and muramyl tripeptide and examined the role of bone marrow transplantation and syngeneic lymphocyte transfers in combination with anti-retroviral therapy. A phase I trial of a candidate AIDS vaccine, gp160 was initiated.

Others:

C. Skerka-Zipfel
J. Balke

Special Volunteer LIR
Howard Hughes Fellow LIR

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00431-04 LIR

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Molecular Biologic Approach to the Immune System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	U.K. Siebenlist	Senior Staff Fellow	LIR, NIAID
Others:	P. Bressler	Medical Staff Fellow	LIR, NIAID
	M.W. Brunvand	National Research Service Award Fellow	LIR, NIAID
	P.F. Zipfel	Guest Researcher	LIR, NIAID
	A.G. Schmidt	Guest Researcher	LIR, NIAID
	J.L. Ambrus, Jr.	Senior Investigator	LIR, NIAID
	A.S. Fauci	Chief	LIR, NIAID

COOPERATING UNITS (if any)

IB, NCI (K. Kelly); FCRF, NCI (U. Rapp).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

6

PROFESSIONAL

5

OTHER:

1

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 ☒ (b) Human tissues
 ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)

We have studied the primary transcriptional response of human peripheral blood T cells to mitogenic activation. Previously we had constructed a subtractive cDNA library and used subtractive and differential screening methods to isolate genes which are induced early on in T cells stimulated with mitogens. The limited number of known inducible genes encode oncogenes, lymphokines and their receptors, i.e., genes with significant roles in proliferation and/or initiation of an immune response. To date we have isolated in excess of 69 induced genes, which include several of the known genes but which represent a much larger number than could have been expected from previous studies. We are pursuing both functional and regulatory aspects of the many novel induced genes. By using mitogenic antibodies to the CD2 and CD3 receptors as well as the co-mitogens anti-CD28, phorbol myristate acetate, D1C8 and ionomycin, we were able to determine that every mitogenic agent induces all genes checked to date but that the co-mitogens could easily distinguish these genes into many different groups with common regulatory elements. Two genes, 464 and 744, behaved similar to interleukin-2 (IL-2). Approximately 1/4 of the genes tested are not expressed in activated human lung fibroblasts, including 464 and 744. These genes are also among those which are very sensitive to the immunosuppressive drug cyclosporin A and among those which are constitutively expressed in human T lymphotropic virus I transformed cells. That these genes possibly encode lymphokines was further supported by their primary sequence, which predicts encoded proteins with typical N-terminal leader sequences and with homologies to each other as well as to a group of so-called inflammatory response genes, a new family of factors which is just emerging. In cell lines permanently transfected with the human immunodeficiency virus *orf-B* protein the induction of some genes, including 464, appears to be negatively affected. In addition to these studies, we have discovered several DNA binding proteins which interact with the mitogen-responsive region of the IL-2 gene. Some of these proteins bind DNA only upon prior activation of the cells. Also, we have delimited further the segment of the c-myc gene, which controls the downregulation of this gene during terminal differentiation. Lastly, we have continued to attempt indirect methods of cloning a B cell growth factor and its receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00361-06 LIR															
PERIOD COVERED October 1, 1987 to September 30, 1988																	
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) International Studies on the Acquired Immunodeficiency Syndrome																	
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: T.C. Quinn</td> <td style="width: 33%;">Senior Investigator</td> <td style="width: 33%;">LIR, NIAID</td> </tr> <tr> <td>Other: A.S. Fauci</td> <td>Chief</td> <td>LIR, NIAID</td> </tr> <tr> <td>C. Brown</td> <td>Expert</td> <td>LIR, NIAID</td> </tr> <tr> <td>H.L. Francis</td> <td>Expert</td> <td>LIR, NIAID</td> </tr> <tr> <td>R. Kline</td> <td>Microbiologist</td> <td>LIR, NIAID</td> </tr> </table>			PI: T.C. Quinn	Senior Investigator	LIR, NIAID	Other: A.S. Fauci	Chief	LIR, NIAID	C. Brown	Expert	LIR, NIAID	H.L. Francis	Expert	LIR, NIAID	R. Kline	Microbiologist	LIR, NIAID
PI: T.C. Quinn	Senior Investigator	LIR, NIAID															
Other: A.S. Fauci	Chief	LIR, NIAID															
C. Brown	Expert	LIR, NIAID															
H.L. Francis	Expert	LIR, NIAID															
R. Kline	Microbiologist	LIR, NIAID															
COOPERATING UNITS (if any) CDC, (R. Ryder); Institute of Tropical Medicine, Antwerp, Belgium, (P. Piot, R. Colebunders); University of Washington, (K. Holmes, J. Kreiss), Johns Hopkins University, (E. Hook, N. Halsey)																	
LAB/BRANCH Laboratory of Immunoregulation																	
SECTION																	
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892																	
TOTAL MAN-YEARS: 2	PROFESSIONAL: 1	OTHER: 1															
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SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The acquired immunodeficiency syndrome (AIDS) has become a global pandemic, with cases reported in 138 countries throughout the world. An intensive research effort has been undertaken in several countries in the Caribbean and Africa to study the unique epidemiologic, virologic, clinical, and immunologic features of AIDS in these areas. In Kinshasa, Zaire, we identified over 10,000 cases of AIDS in 1987 with a male to female ratio of 1:1.5. The disease is predominantly heterosexually transmitted with high seroprevalence rates to the human immunodeficiency virus (HIV) of 3-6% among the general heterosexual population in Kinshasa in contrast to <1% in rural Africa. HIV infection rates of 20-80% have been documented among spouses of AIDS patients, female prostitutes, and among patients attending STD clinics. Similar studies in the U.S. have confirmed a 5% infection rate among patients attending STD clinics with increasing evidence of heterosexual transmission among these patients. The presence of genital ulcerations were significantly associated with heterosexual transmission. In studies of perinatal transmission of HIV infection, over 15,000 pregnant women in Haiti, Kenya, and Zaire, of whom approximately 6% are HIV seropositive have been enrolled in prospective studies. Perinatal transmission which occurs in approximately 50% of children appears to correlate with advanced clinical disease and immunologic impairment of the mother with depressed T4 cells. Perinatal HIV infection was associated with premature birth, low birth weight, and an increased mortality (22%) within the first year of life. Childhood immunization studies on HIV infected children demonstrated efficacy of most vaccines with few adverse effects, documenting the continual need for complete immunization programs. Immunologic studies have confirmed the development of HIV-1 specific antibody dependent cellular cytotoxicity (ADCC) in HIV infected African patients which correlates strongly with the development of antibody to gp41. Further studies will continue to examine the frequency of human retroviral infection (HIV-1, HIV-2, HTLV-1) in selected populations with particular emphasis on natural history, perinatal transmission, and immunopathogenesis which might have relevance to future vaccine studies.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00358-06 LIR

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunopathogenesis of *Chlamydia trachomatis* Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:

T.C. Quinn

Senior Investigator

LIR, NIAID

COOPERATING UNITS (if any)

Johns Hopkins University, (E. Hook, J. Horn)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

1

PROFESSIONAL

1

OTHER:

0

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☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

Chlamydia trachomatis is the most common sexually transmitted bacterial pathogen in the U.S. with an estimated 20 million cases annually. Studies have been in progress to define the clinical spectrum of chlamydial infection, to develop rapid diagnostic assays, and to examine the pathogenesis of chlamydial infections in experimental animal models. In women, chlamydia cervical infection continues to be high (20-30%). Coinfection with gonorrhea and human papillomavirus frequently occur and are strongly associated with the appearance of cervical dysplasia and cervical carcinoma. In a study to determine the reliability of mucopurulent cervicitis (MPC) as a predictive sign for chlamydia infection we found that 51% of patients with MPC had gonococcal cervicitis and 44% had chlamydial cervicitis, while 28% had both. Contrary to previous studies, no significant association between chlamydia and polymorphonuclear leukocytes or with MPC could be established. The positive predictive value of MPC for chlamydial infection was only 44% due to the frequent occurrence of other infections such as gonorrhea and trichomonas. Studies have been initiated to examine the impact of contraceptives on the recurrence of chlamydial infections. In a population with a chlamydia infection rate of 26% at time of enrollment, only 9% demonstrated infection following active use of contraceptives. Diagnostic assays including *in situ* hybridization utilizing DNA and RNA probes for *C. trachomatis* as well as polymerase chain-reaction have been initiated, and a high sensitivity and specificity for detection of chlamydia in tissue samples have been documented. A series of investigations concerning biology and pathogenesis of the TWAR strain of chlamydia are currently in progress. We have demonstrated that optimal growth of TWAR is achieved in HeLa cells following 120 hour incubation, and that TWAR can be detected with monoclonal antibodies directed at the lipopolysaccharide antigen of chlamydia. Inoculation of cynomolgus monkeys with TWAR demonstrated establishment of mucosal infections of the eye, nasopharynx, and intestine, followed by a serologic immune response to the infecting agent. Further studies are planned to examine the clinical spectrum of chlamydia infections and the immunopathogenesis of this pathogen in non-human primate models.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00537-01 LIR
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Immunopathogenic Mechanisms of Human Immunodeficiency Virus Infection</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	A.S. Fauci	Chief LIR, NIAID
Others:	T.M. Folks	Expert LIR, NIAID
	S. Koenig	Senior Staff Fellow LIR, NIAID
	S. Schnittman	Medical Staff Fellow LIR, NIAID
	H.C. Lane	Senior Investigator LIR, NIAID
	L. Vitkovic	Senior Staff Fellow LIR, NIAID
	G. Poli	Visiting Associate LIR, NIAID
COOPERATING UNITS (if any) LMM, NIAID (M. Martin, A. Rabson); LVD, NIAID (B. Moss, P. Earl); LIG, NIAID (T. Kindt, M. Robinson); NCI (C. Fox); Columbia Presbyterian (D. Kotler); NMRI (S. Kessler).		
LAB/BRANCH Laboratory of Immunoregulation		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
11	8	3
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) An intensive effort in LIR was aimed at delineating the <u>immunopathogenic mechanisms</u> of HIV infection as well as the nature of the immune response to <u>HIV</u>. We developed <u>promonocytic</u> and <u>T cell clones</u> which were <u>latently</u> or chronically infected with HIV in order to study the mechanisms of induction of HIV expression. We demonstrated that normal <u>cytokines</u> such as lipopolysaccharide-induced monocyte supernatants were capable of inducing <u>HIV expression</u> in these cell lines. In addition, <u>recombinant TNF-α and TNF-β</u> induced HIV expression in these lines. We further demonstrated at the molecular level that these cytokines induced HIV expression by a <u>transactivating</u> mechanism on the <u>LTR of HIV</u>. We demonstrated that <u>IFN-α</u>, but not AZT was capable of blocking the induction of HIV from these chronically infected cell lines by interfering with the late stage of <u>virus budding</u>, while not interfering with the synthesis of viral proteins. We demonstrated that HIV infects human <u>monocytes</u> in vitro and that the infection is a low-level chronic infection without significant cytopathic effect. In addition, we cultured HIV from monocytes of HIV infected individuals. We demonstrated for the first time that human <u>bone marrow progenitor cells</u> could be infected with HIV. The infected cells were <u>myelomonocytic cells</u>; HIV was produced in large quantities intracellularly in these cells with little virus budding and minimal cytopathic effect. These studies indicate that the bone marrow may serve as an important <u>reservoir</u> of HIV infection and may also explain certain of the <u>hematologic abnormalities</u> seen in some HIV infected individuals. We developed for the first time <u>HIV-specific, MHC-restricted cytotoxic T cell clones</u> from HIV infected individuals. These studies will have important implications in delineating the <u>fine specificity</u> of the cytotoxic T cell response to HIV. Finally, by employing a novel assay system, we demonstrated the presence of antibodies from HIV-infected individuals which <u>block</u> the <u>binding</u> of the envelope protein of HIV to its <u>CD4 cellular receptor</u>.		

Others:

F. Massari
S. Stanley

NRSA
Medical Staff Fellow

LIR, NIAID
LIR, NIAID

LABORATORY OF INFECTIOUS DISEASES
1988 ANNUAL REPORT

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SUMMARY STATEMENT
Annual Report
Laboratory of Infectious Diseases
National Institute of Allergy and Infectious Diseases
October 1, 1987 to September 30, 1988

Despite many new challenges the research goals of the LID remain the same: (a) delineation of the etiology, pathogenesis and epidemiology of medically important viral diseases and (b) development of means for their control. Although the mission of LID has not changed, our research efforts have broadened as advances in molecular biology have provided new technological opportunities to explore medically important viruses at a more basic level. The newer technology has allowed us to define structure, organization and function of a number of viral genomes and to express individual viral proteins which can be evaluated for their role in immunity and usefulness in immunoprophylaxis. Various noncoding regions of the viral genome as well as individual viral proteins are now being evaluated at the molecular level for their role in pathogenesis and virulence. Considerable success has been achieved in each of these areas of research during the past few years, but our overriding concern remains the need to relate these new insights to the understanding and prevention or treatment of viral disease. In this regard, there is cause for optimism that a number of important viral pathogens, such as hepatitis A virus (HAV), respiratory syncytial virus (RSV), parainfluenza type 3 virus (PIV3), dengue virus and rotavirus, may soon be brought under control by immunization with an attenuated viral mutant or a viral protein expressed in a recombinant vector.

The many contributions and achievements of Dr. Robert Purcell, Head, Hepatitis Viruses Section, LID were recognized by the National Academy of Sciences which elected him to membership during their annual meeting in April 1988. This is an honor well deserved.

Molecular Viral Biology

Dengue viruses. Infection by dengue viruses (types 1 through 4) continues to pose a major public health problem in many geographic areas, especially in Southeast Asia, the South Pacific, the Caribbean and in Central and South America. Dengue infection can cause a hemorrhagic fever/shock syndrome which has a high mortality in children and young adults. Unfortunately, there is a lack of specific immunoprophylactic measures against dengue and prevention of dengue infection still depends upon mosquito vector control which is labor-intensive and often ineffective.

The dengue genome consists of a molecule of positive strand RNA approximately 11 kilobases in length which codes for a polyprotein that is apparently cleaved by specific proteases to generate individual viral proteins. The three structural proteins, C, M and E, are located at the amino terminus of the polyprotein while nonstructural proteins NS1-NS2a-N2b-NS3-NS4a-NS4b-NS5 are at the carboxy-terminus.

Envelope glycoprotein epitopes. Linear B-cell epitopes in the envelope (E) glycoprotein of dengue type 4 virus were identified by ELISA using 38 overlapping 15 amino acid peptides that span the 493 amino acid (E) sequence predicted from the nucleotide sequence of cloned DNA. Murine poly- and monoclonal antibodies bound a total of nine peptides. Five of these peptides, all located at the N-terminus of the envelope, were weakly bound. The remaining four, two of which were bound only by monoclonals, exhibited greater reactivity and were

located about 60% of the distance from the N- to C-terminus of the molecule, but were not contiguous with each other. Antibodies in human dengue convalescent sera also frequently recognized these linear epitopes. Future plans include characterization of rabbit anti-peptide antibodies and the use of peptides to define murine and human T cell epitopes (Markoff).

Processing of dengue proteins. Processing of the dengue virus pre-matrix (pM) protein was studied during cell-free translation of RNA transcripts prepared from cloned dengue DNA containing capsid (C), pM, and NH2-terminal envelope glycoprotein (E) sequences. Strategic mutations were produced in C and pM sequences by site-directed mutagenesis of dengue DNA, and mutant RNA transcripts were then translated to elucidate the mechanism for processing. A signal-like function provided by a hydrophobic segment (amino acid residues 100-113 in the dengue polyprotein) at the capsid-pM junction, mediates translocation which is linked to cleavage. Cleavage at the capsid-pM and pM-E junctions is mediated by signal peptidase. Recognition of the "signal" does not require prior cleavage of NH2-terminal capsid sequences. It appears that the "signal" has a polar effect on downstream translocation of E, and possibly nonstructural glycoprotein NS1 sequences (Markoff).

Expression of dengue proteins by vaccinia virus. Because dengue gene expression involves proteolytic cleavage of the polyprotein, a continuous DNA clone was used to achieve expression of dengue genes. The initial goal was to express both protective antigens, E and NS1, from their cloned DNA sequence using vaccinia virus as a vector and then evaluate these antigens for their protective efficacy in experimental immunoprophylaxis.

The three structural proteins, capsid (C), membrane (M) or its precursor PreM, and envelope glycoprotein (E), as well as the nonstructural proteins NS1 and NS2a were expressed from a 5'-terminal fragment of cloned dengue virus DNA. The three dengue virus glycoproteins produced in recombinant vaccinia virus-infected cells were similar to authentic viral glycoproteins produced during dengue virus infection. For example, E was properly glycosylated, but was not expressed on the cell surface. Infection of cotton rats with the vaccinia virus-dengue recombinant induced a poor immune response to the NS1 glycoprotein, while antibodies for the other two glycoproteins were not detected. Nevertheless, mice immunized with this vaccinia virus recombinant developed complete resistance to fatal dengue encephalitis (Zhao, Lai, Bray).

Because of the poor dengue antibody responses to the vaccinia-dengue recombinant an attempt was made to increase immunogenicity by expressing dengue envelope on the surface of infected cells using the respiratory syncytial virus (RSV) G glycoprotein N terminal membrane insertion domain as a carrier. Recombinant DNA constructs were prepared that encoded a truncated N-terminal segment of the RSV G glycoprotein fused to an 85% fragment of the (E) glycoprotein or to smaller E subfragments. Only the latter were expressed on the surface of infected cells, but none of the E fragments induced a detectable E antibody response. Mice immunized with the various vaccinia-chimeric E recombinants were only partially resistant to lethal challenge with dengue type 4 virus and survivors exhibited signs of encephalitis. In contrast, vaccinia recombinants expressing: (i) the 3 structural proteins (C,M,E) plus the first 2 non-structural proteins (NS1,NS2a), or (ii) only the 3 structural proteins, or (iii) only E, induced complete resistance to lethal dengue virus challenge and most surviving mice did not develop signs of encephalitis. These findings together with the observation that dengue E is not expressed on the surface of dengue virus infected cells, indicates that this viral glycoprotein does not require cell surface localization to induce protective immunity. In any case, these three vaccinia recombinants represent promising vaccine candidates for use in prevention of dengue (Bray, Zhao, Lai).

Analysis of the dengue NS1 protein and its use in immunoprophylaxis. The nonstructural protein 1 (NS1) of dengue virus has been shown by others to induce protective immunity in mice. The use of NS1 for immunization against infection has one theoretical advantage over the other dengue protective antigen, envelope (E) glycoprotein. It is thought that antibodies to E cause immune enhancement of disease during subsequent infection by a heterotypic dengue virus. Initially, a series of overlapping oligopeptides that span the entire NS1 amino acid sequence were synthesized. Subsequently, recombinant vaccinia viruses were constructed which expressed: (i) the entire NS1, alone or in combination with other dengue products, or (ii) subfragments of NS1. Analysis of the binding of NS1-specific antibodies to peptides by ELISA and immunoprecipitation of the expressed subfragments of NS1 revealed that the amino terminal third of the NS1 protein contains the major antigenic epitopes. Studies of expression of NS1 by another series of vaccinia recombinants demonstrated that the hydrophobic sequence preceding NS1 is a functional signal for proper processing of this glycoprotein from the polyprotein. In addition, the downstream NS2a sequence was found to be required in cis for the normal cleavage of the NS1/NS2a junction. This observation provides an explanation for the finding that infection with a vaccinia virus-NS1 recombinant which included the entire downstream NS2a sequence induced complete resistance to lethal dengue virus challenge, whereas, a vaccinia virus-NS1 recombinant containing a truncated NS2a sequence induced only partial resistance. This finding has important implications for the incorporation of NS1 into a successful strategy for dengue immunoprophylaxis. Also, it is clear that consideration should be given to using NS1 as the sole antigen in a dengue vaccine (Falgout, Bray, Lai, Chanock).

Dengue E and/or NS1 expressed by a baculovirus recombinant induces highly effective resistance to lethal dengue virus challenge. The high yielding baculovirus-insect cell system was also used successfully to achieve expression of dengue viral proteins from cloned DNA sequences. A recombinant baculovirus containing a 4.0 kilo-base dengue virus cDNA sequence that codes for the three viral structural proteins and nonstructural proteins NS1 and NS2a expressed authentic E and NS1 in infected insect cells. Immunization of mice with these dengue protein products induced complete resistance to fatal dengue encephalitis. Recombinant baculoviruses that expressed only E or NS1 were then constructed to elucidate the basis for this protective immunity. Mice immunized with an infected cell lysate containing either E or NS1 also developed complete resistance to dengue virus challenge (Zhang, Lai, Chanock).

Attempts to construct infectious dengue cDNA. Overlapping cDNA clones were initially identified which spanned the entire dengue 4 sequence of 10,644 nucleotides in length. These separately cloned DNA segments were used to construct a full-length DNA copy. SV40 sequences that contained an early promoter and origin of SV40 DNA replication were inserted into the dengue plasmid and the resulting DNA (SVD4) was used for transfection of COS-1 cells. Recombinant dengue DNA containing an SP6 promoter was also constructed in order to produce RNA transcripts for assay of infectivity in transfected LLCMK₂ cells. Special emphasis was given to removing non-viral sequences from both termini of full-length dengue DNA. The modified SP6-dengue 4 DNA produced precise transcripts in an in vitro system including a cap structure at the 5'-end. Thus far, none of these transcripts has proved to be infectious following the standard transfection assay. Possibly, failure to detect infectivity with cloned DNA resulted from deletion or substitution mutations present in the virus which was cloned or such alterations might have been introduced as an artifact during cloning. A second full-length dengue cDNA copy was constructed from a new set of separate clones. Although the RNA transcripts made from the new clone also failed to show infectivity, these new full-length dengue sequences may prove to be valuable for identifying mutations in the original cDNA and other full length clones by comparison of restriction enzyme sites and sequence among the various clones. In addition, fragments in both non-infectious clones will

be replaced with the corresponding fragments derived from other cloned cDNAs in a continuing attempt to construct a full length cDNA that is infectious (Zhao, Lai).

Hepatitis Viruses

Hepatitis A virus (HAV) immunoprophylaxis. HAV is a picornavirus that was isolated in cell culture for the first time in 1979. HAV was isolated in LID directly from human clinical materials without the intervening serial passage in marmoset monkeys that was necessary for the initial isolation in cell culture. Cell culture-adapted HAV was then thrice cloned by terminal dilution. Since the virus is largely cell-associated and generally is not cytopathic in cell culture, cloning was achieved by terminal dilution and radioimmunoassay. The objective was to develop a live attenuated HAV vaccine suitable for administration to high-risk populations in the United States and abroad. Our strategy involved comparison of the response of susceptible primates (chimpanzees and marmosets) to wild type HAV and to tissue culture adapted mutants of HAV. It quickly became apparent that attenuation of HAV occurs rapidly during serial passage in cell culture and, that achieving the appropriate degree of attenuation would involve trial and error. Because primary African green monkey kidney cells that are free of contamination with latent viruses are difficult to obtain, and because of the recent finding of AIDS-like retroviruses in African green monkeys, the attenuated HAV mutant was adapted to MRC-5 cells, a semi-continuous cell type that is licensed for vaccine production. MRC-5 adapted virus which had been recloned was completely attenuated for chimpanzees and marmosets when inoculated i.v. at a dose of 10^6 TCID₅₀. Plans are underway to begin clinical testing in volunteers in collaboration with the Army (WRAIR). Depending upon whether it is overattenuated or underattenuated for man, "fine-tuning" of the virus will be attempted by introducing deletions and/or insertions into infectious (HAV) cDNA (Purcell, Feinstone).

Molecular biology of HAV. Both the wild type parent and attenuated vaccine candidate HAV have been cloned and sequenced and a full length infectious cDNA clone was derived from the attenuated virus. The relative importance of each of the 24 nucleotide differences between wild type and attenuated viruses in the establishment of attenuation are being evaluated by an analysis of: (a) chimeric infectious cDNAs derived from virulent and attenuated HAV cDNAs and (b) mutants of virulent HAV produced by site specific mutagenesis. The genome of wild type and mutant HAV was divided into three portions: (i) 5' noncoding region, (ii) P1 (capsid region), and (iii) P2-P3 (nonstructural proteins and 3' noncoding region), and all six possible combinations of wild-type and attenuated fragments were constructed. RNA transcripts from three of the cDNA constructs were infectious when transfected into monkey kidney cells. These three chimeric viruses will be inoculated into marmosets to determine which portions of the genome play a role in attenuation. In each of the chimeric cDNAs which were not infectious the P2-P3 regions originated from wild-type virus. This implies that: (a) the wild-type cDNA is not infectious due to a mutation in the P2-P3 region, or more likely, (b) mutations in the P2-P3 region that developed during serial passage in cell culture are responsible for cell culture-adaptation and hence virus with wild-type sequence in this region grows poorly in cell culture (Emerson, Purcell).

Initially all transfections were performed with primary monkey kidney cells. Recently a number of more easily manipulated cell lines (one human and three primate-derived) were successfully transfected with RNA transcribed from the attenuated HAV clone. Using this more rapid transfection assay it was shown that a chimera containing primarily wild-type sequence except for a short region of attenuated sequence in the P2 region was infectious. This short region differs from wild type sequence in only 6 amino acids. Two differences occur in the 2B gene, which has no defined function, while the remaining four changes are in the 2C gene

which is thought to be involved in transcription. Accordingly, site-specific mutagenesis was used to restore individual bases in the attenuated clone to wild-type sequence. The results of transfection assays with these constructs indicated that not more than 4 amino acid substitutions in the wild type 2B and 2C sequence were required for growth in cell culture. Full length cDNAs with selected single point mutations introduced by oligonucleotide site-directed mutagenesis are being used to further delineate the sites responsible for attenuation and to relate altered phenotype to the function of individual genes. Thus, we will begin with a virus of known virulence and genetic sequence and alter its genome step-wise to achieve the ideal level of attenuation (Emerson, Feinstone, Purcell).

Full length HAV cDNAs from wild-type and attenuated HAV HM-175 are also being utilized in an in vitro translation assay to study the genomic organization of HAV. For example, a chimeric construct in which the 5' noncoding region of HAV was replaced with the corresponding region of another picornavirus, encephalomyocarditis virus (EMCV), enhanced greatly the efficiency of in vitro translation. Also, in vitro translation of full length RNA transcripts produced from the HAV cDNAs yielded a broad size range of polypeptides, but these proteins were not immunoprecipitated when reacted with a panel of antibodies to HAV. When the RNA was cleaved at specific sites within the P1 region by digestion with RNase H in the presence of specific DNA oligonucleotides, the major translation product was a polypeptide of the size predicted from correct initiation of translation and termination at the site of cleavage. These results suggest that both internal initiation of translation and appropriate premature termination occurred in this translation system. Translation efficiency of the uncapped genome of other picornaviruses is determined by the structure of the long 5' noncoding region. For this reason efforts are underway to analyze the translation efficiency of HAV RNAs altered in this region and to determine whether a cap structure, added in vitro increases translation efficiency (Emerson, Wychowski).

The 5' terminal HAV gene, codes for a polypeptide only 23 amino acids long. In other picornaviruses VP4 is found in virions and is myristylated. Deletion mutants of HAV were constructed which remove regions of VP4 but leave the first AUG codon and the reading frame correct. These are being studied by transfection assays to determine whether VP4 has a critical function in HAV. In vitro translation will be used to define the exact start of translation, to determine whether VP4 is myristylated, and if so at which site (Emerson, Wychowski, Feinstone).

Biology of HAV. Dr. Lee Maloy prepared antibodies against a synthetic peptide representing the entire predicted 23 amino acids of VP4, and the carboxy terminus of VP2. These antibodies were used to study viral structural proteins by immunoprecipitation and western blot analysis. Because the predicted size for the capsid protein VP4 is only 23 amino acids (about 1/3rd the size of most picornavirus VP4's) it was important to learn whether cleavage of VP0 into VP2 and VP4 actually occurs. Antibodies to VP4 recognized a larger peptide in the 25 to 30 kDa range which is presumably VP0 the uncleaved precursor of VP4 and VP2. A viral VP4 band was not detected in these western blots but the sensitivity of the test for synthetic VP4 was very low (approximately 1 µg of peptide). Antibodies to a synthetic peptide representing the carboxy terminus of capsid protein VP2 recognized 2 closely spaced bands (VP0 and VP2) on western blots. This suggests that cleavage of VP0 to yield VP2 and VP4 does occur but it is possible that the very small VP4 is lost from the mature virion (Feinstone).

Evolution of hepadnaviruses and retroviruses and the identification of a previously unrecognized plus sense open reading frame (ORF). A number of observations suggest that hepatitis B virus (HBV) and retroviruses evolved from a common ancestor: (1) both HBV and retroviruses utilize a unique mechanism of genome replication by reverse transcription of RNA;

(2) highly conserved regions of HBV proteins share considerable homology with retrovirus nucleocapsid, protease, and polymerase proteins; (3) the HBV polymerase shares organizational similarities with the polymerase gene sequence of retroviruses and retrovirus-like genetic elements; (4.) genetic analysis and low stringency blot hybridization indicates that HBV DNA sequences are most closely related to MLV DNA sequences; (5) HBV possesses two previously unrecognized open reading frames which may represent genuine gene sequences. Interestingly, one of the new open reading frames is located on the DNA plus strand of the double-stranded virus genome. This also appears to be a unique feature of retroviruses that encode proteins involved in transcription activation because a plus strand DNA ORF was identified for the first time in the HIV genome, as well as the genomes of related retroviruses of animals. Interestingly, this ORF occurs in a region complementary to the envelope gene sequence and could encode a protein of approximately 190 amino acid residues with a relative molecular mass of 20 kdal if translation began from the first initiation codon. The predicted protein is highly hydrophobic and thus could be membrane associated. Supporting evidence that this ORF represents a genuine gene sequence is that it: (1) is conserved in 12 HIV 1 sequences; (2) is present at the 3' end of the genomes of HIV-related viruses; (3) has conserved 5' and 3' flanking nucleotide sequences essential for transcription; and (4) has a codon periodicity shared by protein-encoding gene sequences. It is possible, therefore, that the HBV and HIV genomes encode unique proteins on a mRNA molecule of opposite polarity to the previously characterized viral transcripts (Miller).

Animal hepadnaviruses: Molecular biology. The woodchuck hepatitis virus (WHV) is an hepadnavirus which is closely related to the human hepatitis B virus (HBV). Infection with each of these viruses is associated with acute and chronic hepatitis and hepatic cell carcinoma in their respective hosts. Thus, the WHV/woodchuck system provides a relevant and convenient model for understanding HBV infections of humans.

The HBV genome contains four long open reading frames encoding the viral core, polymerase and surface proteins as well as a protein of unknown function (X). Sequences at the C-terminus or N-terminus of the WHV X gene were deleted and the resulting mutant DNA genomes were transfected into the liver of adult woodchucks. Wild type WHV DNA, tested as a positive control, initiated a productive infection, whereas the X gene deletion mutants did not. Thus, the X gene product is essential for virus replication (Miller, Chung, Kaneko, Girones).

Analysis of the nucleotide sequence of 4 WHV strains indicated that the level of divergence (1 to 3.5%) was similar to that of HBV strains of the same subtype (1.5 to 2%), but less than that of HBVs of different subtype (8 to 10%). Different regions of the polymerase and X genes would appear to evolve independently and at different rates. Overlapping portions of these genes seem to evolve at a faster rate (with more nucleotide and amino acid variation) than the nonoverlapping portions, despite the constraints placed on them by coding for two different proteins (Miller, Purcell).

The rate at which hepadnaviruses mutate is of interest because these viruses have a propensity to establish persistent infection. The mutation rate of hepadnaviruses, which replicate by reverse transcription of an RNA template should be a function of the fidelity of two (or possibly three) enzymes: the host cell-derived RNA polymerase, and the virus-derived DNA polymerase/reverse transcriptase. Mutation rate was studied by analyzing the nucleotide sequence of: (i) recombinant WHV DNA used to initiate persistent infection of a neonatal woodchuck and (ii) WHV DNA cloned from virus present in serum 16 months post-transfection. Not a single difference was detected suggesting that the mutation rate of WHV is considerably less than that of single strand RNA viruses (Miller, Chung, Kaneko, Girones).

Epidemic non A, non B hepatitis. In 1980 LID scientists described a new form of viral hepatitis caused by a previously unrecognized hepatitis virus with epidemiologic characteristics of HAV. The newly recognized virus: (i) is transmitted by the fecal-oral route, (ii) produces very large water-borne epidemics and (iii) is probably responsible for a significant proportion of endemic hepatitis. This virus is not related antigenically to HAV. The disease, now called epidemic or enterically transmitted non-A, non-B hepatitis (ENANB) is both epidemic and endemic throughout the Indian subcontinent, central Asia, parts of the Middle East and northern Africa, with probable extension to West Africa. The putative etiologic agent, a 27-30nm nonenveloped virus, was first detected in a volunteer study in 1983 and similar virus-like particles have been seen in feces of cases from a number of other outbreaks. The small quantity of such virus-like particles available for study has limited progress. Our initial objective was to confirm the identity of the ENANB virus and to characterize its antigenic and molecular biological properties. Longer term goals include development of: (i) useful assays for diagnosis of infection and seroepidemiologic study and (ii) passive and active immunoprophylaxis for this important human pathogen.

An acute phase stool sample was identified that contained just enough virus-like particles to perform a limited number of serological assays by immune electron microscopy (IEM). IEM analysis of paired sera obtained from epidemic and endemic disease in regions where the virus is prevalent indicated that ENANB hepatitis occurring throughout India, in Pakistan, southern Russia and Algeria is all caused by a single virus or a group of viruses that are closely related. Chimpanzees inoculated with stools containing virus-like particles from the outbreaks developed hepatitis and seroconverted, thus establishing transmissibility to a non-human primate species (Purcell, Kapikian).

AIDS Virus Research

There is a pressing need to define the mechanisms employed by the host to control infection by the human immunodeficiency viruses (HIV). It is especially important to determine whether any of the immune mechanisms which act to suppress HIV could prevent infection if this form of immunity were functionally active prior to exposure of the host to virus. These questions can be addressed most rapidly and most efficiently in experimental animals employing immunodeficiency viruses which closely resemble HIV and which infect and cause AIDS in these species. For these reasons experimental studies of simian immunodeficiency virus (SIV) and feline immunodeficiency virus (FTLV) have been initiated in their natural hosts. These studies are now being performed collaboratively as a part of the AIDS related research contract awarded to Georgetown University. This research program began Aug. 1987. The newly recognized animal immunodeficiency viruses (SIV and FTLV) are being studied intensively using the full range of techniques of contemporary molecular and cellular biology because these viruses have relevance to the natural history of the human immunodeficiency viruses and thus SIV and FTLV can serve as models to investigate mechanisms of disease and immunity in experimental animals. The initial efforts have been directed toward cloning and characterizing the genome of viruses which cause an immunodeficiency syndrome in monkeys (SIV) and cats (FTLV). SIV is genetically closely related to the human AIDS viruses, while FTLV appears to be much less related to the human or primate viruses based on a lack of antigenic cross reactivity (no sequence data is available). These two model systems will allow an in-depth examination (*in vitro* and *in vivo*) of the molecular biology and pathogenesis of these viruses as well as host immune response, and will afford the opportunity for systematic evaluation of various strategies for immunoprophylaxis and antiviral therapy (Purcell, Barnes).

Simian immunodeficiency virus. Three strains of SIV are currently being evaluated: 1) SIV_{delta}, a strain isolated at the Delta Primate Center from an asymptomatic sooty mangabey; 2) SIV_{mac}, a strain isolated from a sick rhesus macaque at New England Primate Center; 3) SIV_{agm}, a strain isolated from an African green monkey at NIH by Drs. Gravel and London (NINCDs). A suspension of SIV_{delta} (prepared by M. Murphy-Corb of the Delta Primate Center) was evaluated for infectivity in rhesus macaques. Only monkeys receiving the 10⁰ or 10⁻¹ dilution were infected and the recipient of the undiluted inoculum is now developing AIDS (Purcell, Johnson, Hirsch, London).

Studies are currently in progress to evaluate cells and cell lines for use in virus titration and neutralization studies. These studies are complicated by the relatively poor cytopathic effect produced by SIV in all transformed CD4-bearing T lymphocyte lines tested thus far. Compounding this problem is the finding that SIV replicates in a limited number of cell lines relative to HIV-1 and HIV-2. To date, the most sensitive cell appears to be the human MT-4 line, which is transformed by HTLV-1. Concurrently, methods are being developed to optimize the sensitivity of these assays (Purcell, Johnson).

A new type of antibody assay is being evaluated that will allow for the rapid screening of large numbers of sera. The assay employs a synthetic peptide representing a highly immunogenic region of the transmembrane glycoprotein (gp 32) as a solid phase ELISA antigen. A similar assay for HIV has proven sensitive and specific. Preliminary studies indicate that the assay will identify SIV specific-antibodies from at least three genera of monkeys regardless of infecting strain of SIV (Johnson, Purcell).

Cloning and sequence analysis have been performed for several isolates of SIV_{mac}. It is clear that isolates obtained from different animals at different times exhibit significant nucleotide sequence variation. Cloning and sequencing SIV_{delta} and SIV_{agm} (a bona fide African green monkey isolate) are underway so that sequence analysis can be extended to additional strains of SIV. Another goal is to obtain disease producing infectious molecular clones that can be used in studies of (i) antigenic drift and (ii) gene function in vivo and in vitro. The env gene (from SIV_{mac} kindly provided by G. Franchini, NCI) has been expressed in a recombinant of the WR strain of vaccinia virus. The expressed product is a biologically active envelope protein that induces syncytium formation in cells susceptible to SIV infection. Most of the macaques infected with the vaccinia-SIV envelope recombinant developed antibodies reactive with the SIV envelope protein. Following a booster infection to obtain maximum antibody titers, the animals will be challenged by SIV_{mac} (homologous strain challenge) given intravenously. A gag-pol construct is currently being prepared in the laboratory of Dr. B. Moss (NIAID/LVD) and will undergo similar evaluation. One of our main goals is to express each gene of SIV in a vaccinia recombinant and to assess its immunogenicity and protective efficacy (Johnson, Hirsch, Purcell).

The ability of SIV to infect human cells parallels the ability of the envelope protein to induce syncytia in these cells. The presence of the CD4 molecule on the cell surface is not sufficient to mediate the entry and subsequent replication of SIV in human cells. Experiments to elucidate the mechanisms of these observations are ongoing (Johnson).

Feline T-lymphotrophic virus. The original FTLV isolate was kindly provided to the laboratory by Drs. N. Pedersen and J. Yamamoto (U.C. Davis). The virus is currently being propagated in two types of cells: 1) feline PBLs that are IL-2 dependent, and 2) Crandell feline kidney cells (CRFK, ATCC CCL 94), an adherent cell line derived from the cortex of the kidney of a 12 week old domestic cat. The fact that this T-lymphotrophic lentivirus will

replicate in other cells (especially adherent cells) make this an attractive model system (Olmsted, Johnson, Purcell).

As with the SIV system, one of the major goals of the program is to obtain disease-producing infectious molecular clones. Since a continuously producing cell line currently is not available, this task will be more difficult for FTLV. One of the main problems will be obtaining enough viral genome to generate cDNA clones to serve as probes for genomic libraries. An alternate approach will be to identify highly conserved sequences among other lentiviruses (in the gag or pol regions) and use these sequences as probes under reduced stringency. In the future, other strains of FTLV will be cloned and sequenced to gain knowledge about the extent and importance of antigenic variation (Olmsted, Purcell).

Epidemiology Section

Rotaviruses are the single most important etiologic agents of severe diarrhea in infants and young children in developed and developing countries. Thus, the need for a rotavirus vaccine is clear. The goal of such a vaccine is to prevent severe rotavirus diarrhea during the first 2 years of life when the disease is most serious.

Live attenuated rotavirus vaccine. We have evaluated an animal rotavirus, rhesus rotavirus (RRV) as a vaccine candidate. RRV has not been recovered under natural conditions from infants or children and thus does not appear to be a virus of the human heritage. In addition, the genes of RRV exhibit significant divergence in sequence from the corresponding genes of the known human rotavirus serotypes. Nonetheless, this virus is similar to human rotavirus type 3 in its neutralization specificity. Furthermore RRV grows efficiently in a semi-continuous line of diploid cells, DBS-FRhl-2. This is an important property because adventitious agents often contaminate primary monkey kidney cell cultures. In addition, RRV can serve as a parent for construction of rotavirus reassortants possessing the major neutralization protein (VP7) of one of the other 3 human rotavirus serotypes. Such reassortants will be needed in a multivalent vaccine since the RRV does not provide broad protection against other human rotavirus serotypes in very young infants (Kapikian, Flores, Chanock).

The safety and antigenicity of orally administered RRV was evaluated in phase I studies initially in adults and subsequently in children of progressively younger age. The vaccine was safe and antigenic in adults and older children. However, in some younger children and infants, it induced a transient febrile response and/or mild transient loose stools. Analysis of pre-vaccination serum neutralizing antibody titers to RRV in several pediatric populations, some with and some without such reactions, suggested that the reactions were related to the absence of pre-existing serum RRV antibodies. Thus, it appeared that pre-existing serum antibodies modified the clinical reaction to the vaccine without significantly affecting its immunogenicity. It should be noted that even in infants lacking serum RRV neutralizing antibodies, RRV was markedly restricted in its replication in the intestinal tract. Unlike human rotaviruses, RRV was rarely shed in a concentration sufficient to be detected by ELISA. Detection of RRV in the stool of vaccinees usually required isolation of virus in tissue culture (Kapikian, Flores).

The previous observations prompted us to evaluate vaccination in the <6 month age group in whom the potential protective effect of maternal antibody might be beneficial in preventing vaccine reactions. In young infants a 10^4 pfu dose of RRV vaccine was not significantly associated with diarrhea defined as 3 or more loose stools within a 24 hour period. Reactogenicity, which characteristically consisted of low grade, self-limited fever, was

considered by parents, physicians and investigators to be an acceptable side effect of vaccination and was not viewed as a basis for terminating clinical trials. Finally, in recent placebo controlled phase I studies in neonates in Venezuela and Burma, the RRV vaccine did not cause reactions but it was shed by most vaccinees and it induced a satisfactory antibody response. The failure of neonates to develop reactions is consistent with our interpretation that maternally-derived serum antibodies in high concentration moderate infection so as to prevent reactions (Kapikian, Flores).

Variable protective efficacy was observed in the 6 trials thus far completed. Most promising were the results from a study in Caracas, Venezuela. In this study in which 151 children 1-10 months of age received the RRV vaccine and the same number received a placebo, thirty episodes of rotavirus diarrhea were detected during the follow-up period, 22 in the placebo group and 8 in the vaccinees for a vaccine efficacy rate of 64% ($P < .01$). Moreover, among 76 vaccinees and 75 controls, 1-4 months of age, vaccine efficacy was 82% ($P < .001$). In addition, for the entire study group, vaccine efficacy was 90% ($P < .01$) for severe rotavirus diarrheal episodes. However, in the Rochester and Arizona trials, vaccine efficacy was nil. Variable efficacy could not be explained by variable immunogenicity because in the Venezuela and Rochester studies seroresponse rates were comparable. In the Venezuela study in which the RRV vaccine was highly effective, 13 of the 23 typeable rotavirus strains associated with diarrhea were serotype 3, the same serotype as the RRV vaccine, whereas in the Rochester study in which the vaccine failed to protect against illness, 29 of 30 rotavirus strains associated with diarrhea were serotype 1, while none were serotype 3. Thus, it appears that serotype specific immunity is necessary for protection against rotavirus diarrhea in the <6 month age group. It should be noted that in older children, the RRV vaccine appears to provide heterotypic protection (in studies in Sweden and Finland). However, this effect may be due to previous priming by natural rotavirus infection followed by a booster effect induced by RRV vaccination (Flores, Kapikian).

Because the Jennerian approach employing the serotype 3 RRV vaccine failed to protect against rotavirus illness in young infants caused by heterotypic strains, we are also pursuing a modified Jennerian approach in an attempt to achieve the needed broad protection. Our modified strategy involves adding RRV reassortants with serotype 1, 2 or 4 neutralization specificity to the vaccine. These reassortants were recovered from the progeny of cell cultures coinfectd with RRV and a human rotavirus under selective pressure of antibody against the former. RRV serves as the donor of attenuating genes, while a human rotavirus of serotype 1, 2 or 4 serves as the donor of a single gene which codes for the major outer capsid neutralization protein, VP7. In this way, single gene substitution reassortants were prepared for human rotavirus serotypes 1, 2, and 4. Each has 10 RRV genes and a single human rotavirus gene which codes for the major neutralization antigen, VP7. In ongoing clinical studies, the reassortants appear to be as attenuated and antigenic as the RRV parent. Currently, intensive efforts are underway to develop a quadrivalent formulation of rotavirus vaccine (with the VP7 of each of the 4 epidemiologically important serotypes) which is safe and which induces antibody to each component. Analysis of neutralizing antibody responses indicates that the RRV VP3 protein represents the major component against which recipients of multivalent vaccine respond. Efforts are underway to increase the neutralizing antibody response to the serotype 1, 2 and 4 VP7 neutralization antigens present in the reassortant viruses by adjusting the dose of the individual viruses in the multivalent formulation. Once the desired formulation is achieved, phase II trials with the multivalent vaccine will be initiated. The laboratory support for many of the phase I and phase II trials continues to be provided by the Epidemiology Section. This includes examination of vaccinees for virus shedding, serotype analysis of rotaviruses recovered from clinical specimens and examination of paired sera for serologic responses to rotavirus (Flores, Kapikian).

Rotavirus: Molecular genetics. Recently a simple dot hybridization assay and a rapid sequencing technique were developed for analysis of sequences within the genome of rotavirus isolates which may have relevance to immunity or virulence. In this context, the sequences in the VP7 gene responsible for serotype specificity of field isolates or the sequence in the VP3 gene related to rotavirus virulence could be identified and related to the particular clinical and epidemiological features of the infections from which the strains were recovered (Flores).

Previous observations on the genomic heterogeneity of field rotaviruses differ from our recent findings of a high degree of stability observed in strains derived from a newborn nursery in Venezuela where nosocomial transmission of asymptomatic infection occurs under conditions which have allowed a virus to persist in the nursery for at least 6 years without significant modification. The VP7 gene of 18 strains recovered at different times from the same nursery over a five year period was sequenced directly from virus present in the stool of neonates. Very few changes in sequence were detected and those changes that occurred did not alter amino acid sequence in most instances. Thus, the rotavirus genome does not undergo genetic drift with high frequency under natural conditions of spread. This is surprising in view of the high rate of spontaneous mutation of rotaviruses estimated from an analysis of neutralization escape mutants selected with neutralizing monoclonal antibodies (Flores).

Previously, it was observed that virulent and naturally attenuated human rotaviruses of all 4 serotypes exhibited sequence dimorphism of the fourth gene which codes for the VP3 outer capsid protein. None of the other 10 rotavirus genes exhibited this association with virulence. The molecular epidemiology of this phenomenon was analyzed further by studying virus shed by newborn babies who underwent symptomatic or asymptomatic infection in Baltimore, MD., Washington, DC, Israel, Chile and Venezuela. For this purpose dot/blot hybridization procedures were developed that permitted differentiation of the fourth gene of virulent strains from that of asymptomatic strains. Thirty-seven additional rotavirus strains recovered from a hospital newborn nursery in which nosocomial transmission of asymptomatic rotavirus infection has persisted for 6 years or more (Maternity Hospital "Concepcion Palacios") as well as 25 specimens collected over a 4 month interval from a newborn nursery in a different hospital (Pediatric Hospital of Caricuao) in Caracas were analyzed. Each of the strains from these two nurseries possessed the conserved fourth gene which had previously been associated with asymptomatic infection of newborns. However, several other strains recovered from asymptomatic newborn babies in other hospitals of the US, Israel and Chile were found to possess a fourth gene related to that of virulent human rotaviruses. The sporadic occurrence of asymptomatic rotavirus infection in the newborn produced by a strain possessing the conserved VP3 sequence of virulent strains suggests that other rotavirus genes as well as host factors also play an important role in expression of virulence (Flores, Kapikian, Chanock).

Single strand cDNA probes prepared by priming the RNA sequence near the hyperdivergent, serotype-specific regions of VP7 of four different human rotaviruses serotypes have allowed us to identify serotype of isolates by hybridization. Over 80 field strains have been "serotyped" by this method which may prove useful in future vaccine trials (Flores).

Variations in sequence of the major neutralization antigen, VP7, of serotype 3 rotaviruses which have a broad host range. The third serotype of human rotavirus shares neutralization specificity with rotaviruses derived from monkeys, dogs, cats, horses, rabbits, and mice. In addition, viruses of this serotype include strains of all known subgroup specificities (i.e., I, II,

neither I nor II, both I and II). The relationships of the major neutralization protein, VP7, of the various serotype 3 strains were analyzed by testing their reactivity with VP7 monoclonal antibodies (mAbs) and by comparing nucleotide and amino acid sequence. This analysis was performed in an attempt to clarify antigenic relationships and to better understand the evolution of serotype 3 rotavirus strains which have established an extraordinarily broad host range. The deduced amino acid sequences indicate an overall homology of more than 85% in the VP7 protein. Among the strains derived from the same species, an even higher degree of homology was observed, but species specific regions were not identified (Nishikawa, Gorziglia).

Sequence analysis of the second rotavirus neutralization antigen, outer capsid protein VP3.

The VP3 genes of additional rotavirus strains were studied by sequence analysis. Surprisingly, the VP3 protein of the porcine Gottfried strain is closely related to the VP3 of human asymptomatic rotavirus strains in various ways including: (1) nucleotide sequence and (2) neutralization by hyperimmune antisera or VP3 cross-reactive neutralizing monoclonal antibodies. The VP3 of Gottfried virus has two cleavage sites which are located in the same positions as in the human strains. Moreover, 5 of the 6 amino acids of the connecting peptide (located between the 2 cleavage sites) are identical to those present in the corresponding site of asymptomatic human rotavirus strains. This finding suggests that the cleavage site is not related to virulence, since the Gottfried virus induces symptomatic infection in piglets. However, it is possible that the determinants of virulence differ in humans and piglets (Gorziglia, Nishikawa).

A comparison of the deduced VP3 amino acid sequence of two virulent bovine NCDV strains and an attenuated NCDV vaccine mutant (RIT 4237), revealed only five amino acid differences which were scattered throughout the protein and did not involve the trypsin cleavage region. One of the virulent strains and the attenuated strain differed in only one amino acid position (residue 76) suggesting that a gene or a series of genes other than the VP3 gene sustained mutations which were responsible for attenuation of the RIT NCDV vaccine strain (Nishikawa, Gorziglia).

Non-group A rotaviruses. The majority of human rotavirus isolates share a common group antigen on the inner of the two capsid shells. These viruses are now known as Group A rotaviruses. The rotavirus vaccines currently in clinical trial possess the Group A antigen. In the early 1980's the isolation of a new group of rotaviruses associated with gastroenteritis in humans and animals was reported. These viruses were morphologically indistinguishable from the previously described Group A rotaviruses, yet they did not possess the previously defined group antigen. Analysis of the viral RNAs of these viruses by gel electrophoresis revealed that, like Group A rotaviruses, their genome contained 11 double-stranded RNA segments. However, the characteristic tight triplet of RNA segments 7-8-9 seen in acrylamide gels was in all cases reduced to a doublet with the displaced RNA segment migrating either more rapidly or slowly, depending on the isolate. Studies by Dr. Malcolm McCrae's group at the University of Warwick (Coventry, United Kingdom) that included gel electrophoresis of the viral genome, terminal RNA fingerprinting, and hybridization analysis demonstrated that non-group A viruses were markedly divergent from Group A rotaviruses.

The failure of non-group A rotaviruses to grow in tissue culture has hampered the development of reagents for epidemiological studies. Efforts are underway to: (i) develop an immunoassay for detection of virus antigen and seroresponses to these viruses, (ii) isolate monoclonal antibodies specific for these viruses, (iii) analyze the genome of non-group A rotaviruses by recombinant DNA technology and (iiii) adapt non-group A rotaviruses to growth in tissue culture, perhaps by gene reassortment with cultivatable non-group A viruses when

these are identified. A bovine Group B rotavirus recently obtained from Dr. Linda Saif at Ohio State University will be used for these initial studies (Green, Kapikian).

Norwalk virus gastroenteritis in the elderly. Norwalk virus, which was initially identified and characterized in LID, is one of a group of small, noncultivable, 27nm viruses which are important etiologic agents of epidemic gastroenteritis in older children and adults. Early epidemiologic surveys in this laboratory using a solid phase RIA, indicated that Norwalk virus was associated with 34% of 70 outbreaks of gastroenteritis that occurred predominantly in the U.S. and developed countries. Norwalk virus has been associated with both food and water-borne epidemics. In the U.S., Norwalk virus infection does not occur frequently in children less than 3 years of age, however, seroepidemiological studies have shown that antibodies to Norwalk or antigenically related viruses are found in more than 50% of adults worldwide.

The development of a biotin-avidin immunoassay for detection of Norwalk virus antigen and for detection of seroresponses to the virus allowed us to perform an extensive survey of gastroenteritis outbreaks in Maryland nursing homes for the elderly. Serologic studies indicated that Norwalk virus was associated with gastroenteritis in approximately one-fifth of the patients; this virus was the single most important agent associated with these outbreaks. Attempts to cultivate Norwalk virus are in progress employing a tissue culture system which has been used successfully for cultivation of a porcine calicivirus. In addition, a porcine calicivirus has recently been obtained which will be used as a model for cloning of the Norwalk virus genome (Green, Kapikian).

Respiratory Viruses

Respiratory syncytial virus (RSV): Analysis of viral genome. RSV is an enveloped RNA-containing virus that is the most important cause of pediatric viral respiratory tract disease. The genome of RSV (vRNA) is a single negative strand of RNA of approximately 15,000 nucleotides. Previously, cDNAs of viral mRNAs of RSV, strain A2, were cloned and sequenced. These studies indicated that RSV encodes ten major mRNAs and ten major viral proteins. Complete nucleotide sequences were determined for 9 of the 10 genes (excepting only the L gene). Sequence analysis of intergenic and flanking gene sequences established the viral gene order (3' NS1(1C)-NS2(1B)-N-P-M-SH(1A)-G-F-M2(22K)-L) as well as the sequences of the intergenic regions. Mapping and sequencing of the 5' end of the L mRNA showed that the L gene overlaps with its upstream neighbor, the M2 gene. Two regions of RSV vRNA remain to be sequenced. One region is the 3' end of vRNA, which by analogy to other paramyxoviruses would be expected to have 50-55 additional nucleotides preceding the start of the NS1(1C) gene and would contain the major viral promoter. The second region includes the L gene and the adjoining 5' end of vRNA. The L gene is estimated to contain 6500 nucleotides, and by analogy to other paramyxoviruses this would be followed by a 5' trailer region of 50-55 nucleotides. The sequence of the first 1010 nucleotides of the L gene has been determined. Also, a 2800 bp cDNA of the L mRNA is currently being analyzed. Preliminary sequence information indicates that this cDNA does not contain a poly(A) tail indicative of the 3' mRNA end, nor does the cDNA overlap with the 1010-nucleotide sequence described above. This indicates that the 2800-bp L cDNA represents an internal segment of the L mRNA (Collins, Spriggs, Stec).

A long term goal is to produce infectious RSV from a full-length cDNA of vRNA. It is thought, by analogy to other paramyxoviruses, that the minimum structural unit sufficient to initiate productive infection is the nucleocapsid. One possible approach to assembling a nucleocapsid from cDNA would be to co-express the nucleocapsid components in tissue culture cells using recombinant vaccinia viruses. For example, cells could be coinfectd with

recombinant vaccinia viruses that express vRNA and the L, N, P and possible other RSV proteins. Alternatively, infectious nucleocapsids could be assembled in vitro by coupled transcription and translation (Collins, Stec).

RSV small hydrophobic (SH) protein: A newly recognized third integral membrane glycoprotein. Detailed analysis of the small SH(1A) protein of human RSV revealed it to be a third integral membrane glycoprotein. The SH protein accumulates intracellularly as four species: two species (4.8K and 7.2K) are unglycosylated and differ in length because translational initiation for the synthesis of the 4.8K species occurs at the second methionine in the sequence. The two other forms (13-15K and 21-30K) contain an N-linked carbohydrate side chain. The 13-15K species appear to be converted into the 21-30K form by post-translational addition of poly-lactosamine to the N-linked side chain. All forms of SH are inserted into intracellular membranes regardless of glycosylation status, and all forms except 4.8K are transported to the cell surface. Protein-mapping studies, including analysis by anti-peptide antisera prepared against the C- or N-terminus of SH, established the orientation of SH in the cell membrane; the C-terminus of the SH protein is extracellular, whereas, the N-terminus is cytoplasmic. The SH protein is unusual among viral glycoproteins because of the multiplicity of intracellular forms and because a large subpopulation of SH protein remains unglycosylated (Olmsted, Collins).

Antigenic fine structure of RSV fusion of glycoprotein. Eighteen neutralizing monoclonal antibodies (MAbs) specific for the fusion (F) glycoprotein were used to construct operational, topologic, and functional epitope maps of this viral envelope glycoprotein. The MAbs defined 16 neutralization epitopes which are organized into three non-overlapping antigenic sites (A,B, and C) and one bridge site (AB). Neutralization and fusion-inhibition tests of the MAbs indicated that antigenic sites A,AB, and C correspond to functional domains of the F protein which are contiguous to the fusion site, whereas, antigenic site B does not appear to be located in close proximity to the fusion site. This interpretation is supported by two additional observations. First, MAb-resistant mutants selected with site B MAbs produce normal sized plaques, whereas, those selected with MAbs that bind to epitopes in sites A and AB produce pinpoint plaques. Second, epitopes in antigenic sites A,AB, and C (which correspond to functional domains) were less variable among RSV clinical isolates than were epitopes in antigenic site B. Sequence analysis of the F genes of antibody-resistant mutants will allow us to identify amino acid residues which: (i) are involved in binding neutralizing antibodies and (ii) are in close proximity to the fusion site (Coelingh, Beeler).

Antigenic analyses of RSV G glycoprotein, the attachment protein. The RSV G protein contains 298 amino acids (strain A2), is anchored in the membrane by a hydrophobic, N-proximal domain, and contains a large content of N-linked and O-linked carbohydrate. Previously, it was shown that truncated forms of the G protein (expressed from engineered cDNAs) that contained only the N-terminal 230 (G-230), 180 (G-180) or 71 (G-71) amino acids of the parental protein were inserted into membrane, glycosylated and transported to the cell surface. This indicated that most of the ectodomain was not required for these activities. During the past year, vaccinia virus recombinants that express individually the truncated forms described above were used to immunize cotton rats in parallel with vaccinia-G (expressing the parental protein) to compare their capacity to induce protein-binding serum antibodies, RSV-neutralizing serum antibodies, and resistance to RSV infection. G71 and G180 were neither immunogenic nor able to induce resistance. This was not surprising in the case of G71 because the truncated protein lacks most of the G ectodomain. The lack of immunogenicity of G180 could be due to either: (i) the removal by the truncation of the major antigenic sites in the G protein, or (ii) the indirect alteration of antigenic sites due to conformational changes induced by the truncation. The levels of serum neutralizing antibodies and resistance to RSV

infection induced by G230 were equal to or greater than those observed for parental G protein. This indicated that the C-terminal 20% of the G protein is not required for the induction of protective immunity (Collins, Olmsted).

RSV immunoprophylaxis: Obstacle of immunosuppression by maternal antibodies. Young infants, who universally possess maternally derived RSV antibodies, mount a relatively poor antibody response to RSV glycoproteins following infection despite extensive replication of RSV. Analysis of this phenomenon in cotton rats revealed that cotton rat RSV hyperimmune antiserum, passively transferred to animals prior to intradermal infection with vaccinia recombinant viruses expressing the RSV envelope glycoproteins, suppressed the antibody response to the RSV glycoproteins but not to vaccinia virus antigens. The cotton rats which had their immune response suppressed by passively transferred antibodies (that were equivalent in titer to RSV antibodies present in the serum of one to four month old human infants) were more susceptible to infection with RSV than animals inoculated with control serum lacking RSV antibodies. Furthermore, many of the immunosuppressed animals infected with the vaccinia recombinant viruses developed antibodies to the RSV glycoproteins which had abnormally low neutralizing activity. Thus, pre-existing serum RSV antibodies had dramatic quantitative and qualitative effects on the immune response to RSV glycoproteins that may explain, in part, the poor RSV antibody response of young human infants to infection with RSV. The suppressive effect of passively administered RSV immune serum described above could be largely overcome by administering recombinant vaccinia-RSV by the topical intranasal route. Also, immunization with a large quantity of purified F glycoprotein (5µg in alum) was able to overcome immune suppression by passive antibodies. Our observations suggest that immunosuppression by pre-existing, passively acquired RSV antibodies may constitute a major obstacle to immunoprophylaxis against RSV during early infancy when immunization is most needed (Murphy, Olmsted, Collins, Chanock).

RSV immunoprophylaxis: Safety and efficacy of purified F and G glycoproteins. It was shown previously that immunization with formalin-inactivated RSV potentiated pulmonary disease in humans or cotton rats during subsequent infection with RSV. Thus, the possibility of disease-potential must be addressed for each new paramyxovirus vaccine. In the present study, the immunogenicity, efficacy, and disease potentiating ability of a purified RSV F and G glycoprotein vaccine and a formalin-inactivated RSV were compared over a wide dosage range. It was observed that: 1) the purified F and G glycoprotein vaccine was highly immunogenic; 2) a high level of resistance to pulmonary virus replication was induced even in instances in which relatively low levels of serum neutralizing antibodies developed; 3) the purified F and G vaccine did not potentiate disease at any dose, whereas intermediate doses of formalin-inactivated RSV vaccine (but not high or low doses) potentiated RSV disease, and 4) both the formalin-inactivated RSV and purified F and G glycoprotein vaccines induced a high level of antibodies capable of binding to purified RSV glycoproteins, but these antibodies exhibited a significantly reduced capacity to neutralize infectivity of RSV. These results are encouraging because they suggest that purified paramyxovirus glycoproteins might be used safely as a vaccine. However, it would be desirable to develop a glycoprotein vaccine that induces antibodies with high neutralizing capacity that more closely approximate the antibody response stimulated by natural infection (Murphy, Prince).

RSV immunoprophylaxis: Efficacy of vaccinia-RSV G and F recombinants in monkeys. Vaccinia recombinant viruses containing the attachment (G) or fusion (F) glycoprotein of RSV were evaluated for protective efficacy in owl monkeys. Previous studies in a variety of new and old world monkeys demonstrated the safety and immunogenicity of the recombinants. Owl monkeys infected intradermally with vaccinia-F and vaccinia-G developed a high titer of serum neutralizing antibodies which exceeded that of other owl monkeys infected with RSV.

Resistance of vaccinia-F and vaccinia-G immunized monkeys to subsequent challenge with RSV was complete in the lungs and almost complete in the upper respiratory tract. These results are encouraging because they demonstrate that vaccinia recombinant viruses administered intradermally can provide a high level of protection for primates against a virus that replicates almost exclusively on the epithelial surface of the respiratory tract (Olmsted, Collins, Murphy).

The vaccinia recombinants employed in these studies (as well as similar studies of parainfluenza type 3 virus glycoproteins) were constructed from the WR strain of vaccinia which is a highly neurotropic mutant, clearly not suitable for use in humans. Because of the encouraging results obtained in owl monkeys with vaccinia-RSV recombinants, similar vaccinia-RSV recombinants are now being constructed from vaccinia strains which have a long history of safe use in humans. The new recombinants will be evaluated in clinical trials following demonstration of safety and efficacy in experimental primates (Collins, Spriggs).

Safety of vaccinia recombinants. Because live recombinant vaccinia viruses might cause disseminated disease and extensive local lesions in immunocompromised individuals, it may be necessary to further attenuate vaccinia without adversely affecting its immunogenicity before it can be considered for use in humans. It was recently shown by Drs. Flexner and Moss (LVD) that co-expression of lymphokine IL-2 attenuates vaccinia recombinants for mice and rabbits without significant loss of immunogenicity. In studies performed in collaboration with Drs. Flexner and Moss, we sought to determine if this is also true for non-human primates. The observations made in patas and rhesus monkeys indicate that co-expression of the IL-2 gene by a vaccinia virus can decrease lesion size as well as the time required for resolution of the lesion without significant diminution of immunogenicity (Murphy).

Parainfluenza type 3 virus (PIV3): Analysis of viral genome and its products. Human parainfluenza virus type 3 (PIV3), a paramyxovirus, is second in importance only to RSV as a major etiologic agent of pediatric viral respiratory tract disease. Previously, PIV3 was shown to encode six structural proteins: the HN (69-70 Kdal) and F (60-63 Kdal) glycoproteins, which constitute the virion envelope spikes; the M protein (35-40 Kdal) which underlies the lipid envelope; and the NP (66-68 Kdal), P (83-Ddal) and L (200 Kdal) proteins, which associate with RNA to form the nucleocapsid. PIV3 also has a single non-structural protein C (22 Kdal) that was shown to be encoded by a second open reading frame in the P+C mRNA.

The role of individual PIV3 proteins in host immunity was investigated by expressing the cDNA of the F, HN or NP mRNA in a vaccinia recombinant virus. Recombinants expressing the HN or F protein induced high levels of protective immunity in rodents and nonhuman primates. This demonstrated that a single intradermal inoculation of vaccinia-PIV3 F and HN can induce immunity in primates against a virus that replicates in the respiratory tract. This also established that both HN and F are important neutralization antigens, and demonstrated the feasibility of a subunit vaccine for PIV3. In a continuation of this work, we are constructing analogous recombinant vaccinia viruses using parental vaccinia strains (Wyeth and Lister) that are suitable for use in humans. Also, recombinant adenoviruses that will express individually the HN and F proteins are under construction (Spriggs, Collins).

PIV3 HN surface glycoprotein: Synthesis and maturation. The intracellular synthesis and maturation of the hemagglutinin-neuraminidase (HN) protein was investigated by the expression of cDNAs that had been engineered to encode N-terminally truncated HN proteins. The truncated HN proteins contained progressively larger deletions of the N-terminal cytoplasmic and transmembrane domains. Analysis of deletion mutants indicated that the folding of the HN ectodomain into a biologically active and conformationally authentic form occurs to

completion in the rough endoplasmic reticulum. However, the finding that two biologically-active HN deletion mutants remained localized primarily in the rough endoplasmic reticulum indicated that correct folding was not the sole prerequisite for movement through the exocytotic pathway. Finally, the glycosylation of the HN protein (which contains only N-linked sugars) and the respiratory syncytial virus G protein (which contains both N-linked and O-linked sugars) was compared. By cDNA mutagenesis and expression, chimeric proteins were expressed that consisted of the N-terminal cytoplasmic and membrane domains of each protein fused to the ectodomain of the other. The processing and maturation of each ectodomain was observed to be independent of the origin of the cytoplasmic and membrane domains. Thus, the signals for processing and maturation reside in the ectodomain of these disparate glycoproteins (Spriggs, Collins).

PIV3 HN glycoprotein: Structural and antigenic characterization. Baculovirus recombinants expressing the human PIV3 HN protein were constructed to extend our previous antigenic and functional characterization of the HN to include structural analysis. The HN protein expressed by the initial baculovirus recombinants were shown to be glycosylated, biologically active, immunogenic, and antigenically authentic, but was produced in relatively small quantities. A second generation of recombinants was constructed which express HN as a fusion product. These new recombinants achieve a level of expression which is 20-fold higher than our initial recombinants. Recombinant HN protein will be purified prior to structural analysis by x-ray crystallography (Coelingh).

PIV3 fusion glycoprotein: Antigenic analysis. To extend our antigenic and functional analysis of the PIV3 surface glycoproteins, monoclonal antibodies (MAbs) were produced to the fusion (F) glycoprotein. These MAbs define 20 epitopes, 14 of which participate in neutralization. The neutralization epitopes are distributed among three non-overlapping antigenic sites (A,B, and C) and one bridge site (AB), while the six non-neutralization epitopes form four distinct antigenic sites. Analysis of the biological activities of MAbs indicated that antigenic sites AB,B, and C correspond to functional domains of the F protein. Antigenic variants have been selected using neutralizing and fusion-inhibiting MAbs, and sequence analysis of these variants and of naturally-occurring clinical PIV3 isolates is currently in progress. These studies will identify amino acid residues involved in neutralization by antibodies as well as residues important for fusion function (Coelingh).

Influenza B virus: Evaluation of a ca reassortant for attenuation and efficacy. Influenza B virus causes significant morbidity in infants and children and excess mortality in the elderly and high risk persons which could be prevented by immunization. Dr. John Maassab at the University of Michigan School of Public Health has developed an attenuated cold adapted (ca) donor virus (influenza B/Ann Arbor/1/66) by serial passage of virus in primary chick kidney tissue at 25°C, a temperature suboptimal for replication of wild type virus. This ca virus has sustained attenuating mutations in one or more of its "internal genes"; these are genes that code for non-surface proteins and hence can be transferred by gene reassortment to new variants of influenza B virus that arise in nature and pose an epidemic threat. The attenuated ca donor virus was mated with a virulent wild type virus (B/Texas/84) and a reassortant was isolated that derived its HA and NA genes from the wild type virus and the remaining six RNA segments ("internal genes") from the attenuated donor virus.

The ca reassortant influenza B virus was restricted in replication in the upper and lower respiratory tract of hamsters and chimpanzees compared to its wild type influenza B virus parent. Isolates of the influenza B ca reassortant recovered after up to 15 days of replication in immunosuppressed hamsters did not exhibit instability of the ts or attenuation phenotype as indicated by tests performed in cell culture and in hamsters. Our observations concerning

restriction of virus replication in the lower respiratory tract of animals, as well as phenotypic stability and immunogenicity of this candidate virus vaccine provides a basis for larger scale studies in fully susceptible adults as well as infants and children. Recently, we evaluated the safety, attenuation, immunogenicity, infectivity, and efficacy of a ca B/Ann Arbor/1/86 reassortant virus in seronegative adult volunteers. The reassortant was: (i) restricted in its replication in these susceptible adults, (ii) satisfactorily attenuated, (iii) immunogenic and (iiii) able to induce resistance to illness caused by wild type influenza B virus (Snyder, Murphy).

Avian-human (AH) influenza A virus reassortants: Comparison of different avian influenza A virus donor strains. We previously demonstrated that live influenza A reassortant viruses which derive their genes coding for surface glycoproteins from a human wild-type influenza A H3N2 or H1N1 virus and the remaining six genes from the avian influenza A/Mallard/NY/6750/78 (H2N2) donor virus were safe, attenuated, immunogenic, and efficacious in seronegative adults. Two other avian influenza viruses, A/Pintail/Alberta/119/79 (H4N8) and A/Mallard/Alberta/88/76 (H3N2), were also recently evaluated as donors of attenuating genes. An AH reassortant prepared from the A/Pintail/79 virus appeared overattenuated, whereas, the A/Mallard//Alberta/76 donor virus yielded an AH reassortant which was as attenuated and as immunogenic over a broad range of doses as a comparable A/Mallard/NY/78 reassortant virus. Because the A/Mallard/Alberta/76 donor virus did not appear to offer a demonstrable advantage, future efforts will be directed toward the further evaluation of reassortants derived from the more thoroughly characterized A/Mallard/NY/6750/78 donor virus (Snyder, Murphy).

AH Reassortant viruses: Stability of avian influenza A virus genes that play a major role in attenuation. The M and NP genes of the avian influenza A/Mallard/78 donor virus play a major role in attenuation of AH influenza reassortants for primates. These genes, which exhibit significant sequence divergence from the corresponding human influenza A virus genes, were examined for stability of phenotype and genotype by subjecting a single gene reassortant virus, which contains either the A/Mallard/78 virus M or NP gene in combination with 7 genes from the wild type human influenza A/Udorn/72 (H3N2) virus, to serial passage in squirrel monkeys. After 3 such passages neither reassortant exhibited an alteration in host range restriction for primate lung. This property will be retested after several additional serial passages. At that time we will also compare the nucleotide and deduced amino acid sequences of the avian influenza M and NP genes of the input and serially passaged reassortants. If a change in phenotype occurs, this analysis may allow us to identify the sequences responsible for host range restriction of these genes in primates. In any case, the stress of serial passage in primate lung constitutes the most stringent test for phenotype stability that we can perform at this time (Snyder, Murphy).

Comparison of AH and ca reassortant influenza A viruses in fully susceptible infants and young children. Previously avian-human (AH) and ca reassortant influenza A viruses prepared from the same H1N1 and H3N2 wild type influenza A viruses were compared in side by side tests in seronegative adult volunteers. The two types of reassortant viruses were similar with respect to safety, attenuation, infectivity, immunogenicity and protective efficacy. Neither form of reassortant virus appeared to offer an advantage over the other. For this reason we extended our analysis of these reassortants to fully susceptible infants and young children, 6 to 48 months of age. This age group may prove to be the population in which live influenza A virus vaccines are most useful because these attenuated viruses are most infectious for individuals who lack prior experience with influenza A viruses. Reassortant viruses were derived from the wild type human influenza A/Bethesda/1/85 (H3N2) or influenza A/Kawasaki/9/86 (H1N1) virus. The ca and AH reassortants derived from the same wild virus type donor were similar with respect to attenuation, immunogenicity, infectivity and efficacy against challenge with homologous attenuated virus. Of interest, both AH and ca H1N1

reassortants appeared to be more infectious than their H3N2 counterparts. A single infection induced complete resistance to subsequent challenge with the homologous attenuated virus. However, 55-60% of vaccinees developed a significant antibody response to a second dose of vaccine (Snyder, Murphy).

ca Reassortant influenza A viruses: Stability of attenuating genes. Attempts are underway to isolate phenotypic revertants of previously characterized influenza A virus reassortants which derived only one gene from the influenza A/Ann Arbor/60 ca donor virus, while the remaining 7 genes were derived from a wild type influenza A virus. The single ca gene reassortants analyzed were those containing a ca gene known to contribute to the attenuation of vaccine candidate ca reassortants. Each of four ts⁺ revertants of a single gene PB2 reassortant virus also regained the ability to replicate in the lower respiratory tract of hamsters indicating that attenuation specified by the ca PB2 gene is closely linked to the ts phenotype. Phenotypic revertants of single gene PB1 and M reassortant viruses have also been isolated and are currently under evaluation (Treanor, Snyder, Murphy).

Sequence dimorphism of avian influenza A virus NS gene: Possible application to development of live attenuated virus vaccines. The NS gene of avian influenza A viruses exhibits sequence dimorphism, i.e., there are two allelic forms of the NS gene, allele A or allele B. One of these alleles (A) is also present in human influenza A viruses, while the other avian influenza A virus NS allele (B) is highly divergent from the corresponding gene of the human influenza A viruses. The NS gene B allele may be restricted in its function in primate cells because of its marked divergence from the NS gene A allele of human influenza A viruses. This possibility is now being examined using reassortant viruses which contain the NS gene B allele (of avian influenza origin) and 7 genes derived from a human influenza A virus. The level of replication of these reassortant viruses in squirrel monkeys will be compared with that of a human wild type influenza A virus to determine if the B allele specifies host range restriction in primate respiratory tissue (Snyder, Murphy).

HONORS AND ADWARDS

Robert M. Chanock

Invited speaker, "Live viral vaccines for respiratory and enteric tract diseases" Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Nov 24, 1987.

Invited speaker, "Epidemiology of respiratory syncytial viruses" Children's Hospital, Harvard Medical School, Boston, Mass., Nov. 25, 1987.

Participated in annual meeting of Board of Scientific Governors of the Research Institute of Scripps Clinic, La Jolla, CA, Jan. 13, 1988.

Program chairman, Wallace Rowe Fourth Annual NIAID Symposium on Animal Virology: Viral pathogenesis, Feb. 1-2, 1988.

Participated in planning meeting at Rockefeller Univ., NY, Mar. 16, 1988 which was held to establish agenda for second bilateral meeting of Delegations of Soviet National Academy of Sciences and U.S. National Academy of Sciences. Topic: Prevention of Use of Biological Weapons.

Invited speaker, Centennial Celebration of American Pediatric Society, May 3, 1988. Presented lecture "Control of pediatric viral diseases: past successes and future prospects."

Member of delegation of U.S. National Academy of Sciences, Committee on International Security and Arms Control, Subcommittee on Biological Weapons, which met with delegation of the Academy of Sciences of the U.S.S.R. to discuss Prevention of Use of Biological Weapons, May 9, 1988, Washington, D.C.

Participated in business meeting of Section on Medical Microbiology and Immunology, National Academy of Sciences, April 25, 1988.

Albert Z. Kapikian

Invited to be chairman of session on "Virology I" on September 10, 1987, at Cold Spring Harbor Laboratory Conference on "Modern Approches to New Vaccines Including Prevention of AIDS," Cold Spring Harbor, New York.

Invited as Temporary Adviser to the 4th Meeting of the Scientific Working Group on Immunology, Microbiology and Vaccine Development of the Diarrheal Diseases Control Program of the World Health Organization, September 22-25, 1987 in Geneva, Switzerland. Dr. Kapikian is a member of this Scientific Working Group.

Invited speaker at Symposium of Joint Immunology Boards and Viral Diseases Panels of U.S. - Japan Cooperative Medical Sciences Program, December 2, 1987 in Monterey, California. Presentation on "Viral Gastroenteritis."

Co-chairman (with Dr. Konno) of Viral Gastroenteritis session on December 3, 1987 of Scientific Program of U.S. - Japan Cooperative Medical Sciences Program Working Conference on Viral Diseases, December 3-4, 1987 in Monterey, California.

- Invited speaker at NIAID Planning Session with the Director, NIH. Presentation on Development of a Rotavirus Vaccine, January 11, 1988, Building 1, NIH, Bethesda, Maryland.
- Invited as temporary adviser to the Fifth Meeting of the Scientific Working Group on Immunology, Microbiology and Vaccine Development of the Diarrheal Diseases Control Program of the World Health Organization, March 1-4, 1988 in Geneva, Switzerland. Dr. Kapikian is a member of the Scientific Working Group.
- Invited speaker at Oral Immunization Symposium in Birmingham, Alabama, March 21-22, 1988. Presentation on immunity to rotaviruses on March 21, 1988 (with Dr. Edelman).
- Invited speaker at Frontiers in Infectious Diseases Symposium, "Viral chemotherapy, vaccines and interactions with the host immune system," April 24-26, 1988 in Brompton Hall (London suburb), England. Presentation on Rotavirus in session on New Viral Vaccines, April 26, 1988.
- Invited speaker at International Symposium on the Immune Response to Viral Infections, April 28-29, 1988, in Florence, Italy. Presentation on "Strategies for the development of a rotavirus vaccine against infantile diarrhea with an update on clinical trials of rotavirus vaccines" on April 28, 1988.
- Invited to be co-chairman of session on Acute Viral Infections on April 28, 1988 at International Symposium on "The Immune Response to Viral Infections," April 28-29, 1988 in Florence, Italy.
- Invited speaker at seminar "Oral Vaccines," American Society for Microbiology Annual Meeting, May 8-13, 1988, in Miami, Florida. Presentation on Rotavirus Vaccines, May 9, 1988.
- Invited as Temporary adviser to World Health Organization Diarrheal Disease Control Program to make site visit to Dept. of Medical Research, Ministry of Health, Rangoon, Burma, May 30-June 2, 1988 to review plans for a field trial of rotavirus vaccine among newborn infants.
- Invited (while in Rangoon) to give lecture on viral gastroenteritis at Dept. of Medical Research, Ministry of Health, Rangoon, Burma, June 1, 1988.
- Invited as temporary adviser to World Health Organization Diarrheal Diseases Control Program to make site visit to Vaccine Trial Center, Mahedol University, Bangkok, Thailand to discuss rotavirus vaccine study in infants, June 3, 1988.

Robert H. Purcell

- Invited speaker, Workshop on Hepadnavirus Animal Models, Bethesda, MD, Sept. 21-22, 1987.
- Invited speaker, Symposium on Australia Antigen and AIDS. Castro-Caro, Italy, Sept. 29-30, 1987.
- Invited speaker, Third annual Conference on Military Medicine, Bethesda, MD, Oct. 26-27, 1987.
- Invited participant, Technical Advisory Group, Geneva, Switzerland, Nov. 2-5, 1987.

Invited speaker, Scientific meeting on Control of Hepatitis B Infections in Infants and Children, Whakatane, N.Z., Nov. 12-13, 1987.

Invited speaker, Symposium on Control of hepatitis B, Hamilton, N.Z., Nov. 17, 1987.

Invited speaker, Wallace Rowe Fourth Annual NIAID Symposium, Bethesda, MD, Feb. 1-2, 1988.

Invited speaker and member of panel, U.S.-Japan Bilateral Science Agreement, Panel on Hepatitis, LaJolla, CA, Mar. 16-17, 1988.

Invited participant, Symposium on Transfusion Associated Infections and Immune Response, Mar. 20-22, 1988.

Invited speaker and member of Advisory Group, Stanford Medical School, Palo Alto, CA, March 23, 1988.

Invited speaker, Grand Rounds, NIH, Bethesda, MD, Mar. 30, 1988.

Invited speaker, Grand Rounds, NIH, Bethesda, MD, June 1, 1988.

Invited participant, USPHS meeting on AIDS, Charlottesville, VA, June 2-3, 1988.

Invited participant, Symposium on Liver Cell Cancer, Freiburg, Germany, June 5-8, 1988.

Elected to the U.S. National Academy of Sciences, 1988.

Brian R. Murphy

Invited speaker, Orthomyxovirus Symposium held at VII International Congress of Virology, Edmonton, Canada, August 1987.

Invited to chair workshop, "Respiratory Viruses", Duke University Medical Center, Durham, NC, Feb. 13, 1988.

Invited speaker at the "Oral Immunization Symposium" Birmingham, Ala., Mar. 21, 1988.

Invited speaker, Stanford University's lecture series on "Molecular and Genetic Medicine - Molecular Responses to Virus Infection", May 2, 1988.

Recipient of United States Public Health Service Outstanding Service Medal, June 6, 1988.

Ching-Juh Lai

Invited speaker, Bureau of Biologics, FDA, Bethesda, MD, Feb. 22, 1988.

Invited speaker, Contractors Meeting for Flavivirus Research, Walter Reed Army Institute of Research, Washington, D.C., Mar. 1, 1988.

Invited speaker, Bureau of Disease Control, Department of Health, The Executive Yuan, Taiwan, June 5-13, 1988.

Invited speaker, American Society of Virology meeting, Austin, TX, June 12-16, 1988.

Invited speaker, U.S.-Japan Joint Viral Diseases Conference, Tokyo, Japan, July 18-20, 1988.

Invited speaker, Modern Approaches to New Vaccines meeting, Cold Spring Harbor Laboratory, New York, Sept. 14-18, 1988.

Michael Bray

Invited speaker, Contractors Meeting for Flavivirus Research, WRAIR, Washington, D.C., March 1, 1988.

Invited speaker, meeting of Modern Approaches to New Vaccines, Cold Spring Harbor Laboratory, New York, Sept. 14, 1988.

Suzanne U. Emerson

Invited speaker, "In vitro analyses of vesicular stomatitis virus RNA synthesis" Laboratory of Biochemical Pharmacology, National Institute of Diabetes and Digestive and Kidney Diseases, Feb. 1988.

Invited speaker, Conference on Hepatitis A Virus and HAV Vaccine Development, WRAIR, Washington, D.C., "Growth in cell culture of HAV from infectious cDNA", March 1988.

Invited speaker, Wagner Symposium, Charlottesville, VA, "Constructing a hepatitis A virus that can be studied", May 1988.

Stephen M. Feinstone

Invited to deliver seminar, Cetus Corp., May 3, 1988. "Hepatitis A virus."

Jorge Flores

Poster presentation at American Society of Virology, June 1988.

Invited speaker, Japan Viral Diseases Conference, Tokyo, Japan, July 18-20, 1988.
Presentations: Molecular epidemiology of the rotavirus gene 4; A dot hybridization using amplified DNA probes for rotavirus serotyping.

International Virology Meeting, Edmonton, Canada, August 1988. Poster presentation: Clinical trials with the rhesus rotavirus vaccine and its reassortants.

"Luis Razzetti Honorable Mention" from the Venezuelan Medical Federation for work on: Antigenicity and reactogenicity of the rhesus rotavirus vaccine MMU 18006 in Venezuelan newborn infants. Caracas, Venezuela, March 1988.

Invited speaker, Third NIH Rotavirus Workshop, NIH, Bethesda, MD, Sept. 8-9, 1988. Presentations: NIH reassortant vaccine trials; Use of nursery strains as potential rotavirus vaccines; DNA probes for serotyping rotavirus in stool.

Mario Gorziglia

Presentation American Society of Virology meeting, June 1988.

Lewis Markoff

Invited speaker, Contractors Meeting on Dengue Vaccine Development, WRAIR, Washington, D.C., March 1988.

Invited speaker, American Society for Virology, Austin, TX, June 1988.

PHS Outstanding Achievement Award, June 1988.

Roger H. Miller

Invited speaker, "Evolutionary relatedness of hepatitis B virus and retroviruses" Albert Einstein College of Medicine of Yeshiva Univ., New York, Jan. 13, 1988.

Invited to give seminar "X region specific transcript in woodchuck hepatitis virus infected liver" Fox Chase Cancer Center, Phila., PA, July 21, 1988.

Kazuo Nishikawa

Presentation at American Society of Virology meeting, June 1988.

Mark H. Snyder

Invited to participate and present "Influenza an update" Georgetown University School of Medicine, Feb. 9, 1988.

Invited to present lecture, Division of Infectious Diseases, Albany Medical College, Albany, NY, April 25, 1988. "Genetic basis of attenuation of live influenza A virus vaccines".

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00308-07 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>In Vitro and In Vivo Studies of Hepatitis A Virus</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Robert Purcell, M.D.	Head, HV Section LID, NIAID
Others:	Stephen M. Feinstone, M.D. Medical Officer	LID, NIAID
COOPERATING UNITS (if any) Smith Kline RIT (d'Hondt); FDA (Daemer), Fairfield Hospital, Melbourne, Australia (Gust)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Hepatitis Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.7	.4	.3
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The hepatitis A virus (HAV) is a picornavirus that was isolated in cell culture for the first time in 1979. We have succeeded in isolating virus directly from human clinical materials without the intervening serial passage in marmoset monkeys that was necessary for the initial isolation in cell culture. Cell culture-adapted HAV was thrice cloned by terminal dilution. Since the virus is largely cell-associated and generally is not cytopathic in cell culture, cloning was achieved by terminal dilution and radioimmunoassay. The objective of this project is to develop a live attenuated hepatitis A vaccine suitable for administration to high-risk populations in the United States and abroad. Our strategy involves comparison of the response of susceptible primates (chimpanzees and marmosets) to wild type HAV and to tissue culture adapted mutants of HAV. Attenuation of HAV occurs rapidly during serial passage in cell culture and, initially, the appropriate degree of attenuation must be determined by trial and error. Because primary African green monkey kidney cells that are free of contamination with latent viruses are difficult to obtain, and because of the recent finding of AIDS-like retroviruses in African green monkeys, we have elected to adapt the attenuated HAV to MRC-5 cells, a semi-continuous cell type that is also licensed for vaccine development. Adaptation and recloning of the virus have been achieved and retesting of the virus in primates is in progress. The MRC-5 adapted virus was completely attenuated for chimpanzees and marmosets when inoculated i.v. at a dose of 10^9 TCID₅₀. Plans are underway to begin clinical testing in volunteers in collaboration with the Army (WRAIR).		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00309-07 LID

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Hepatitis A Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Stephen M. Feinstone, M.D. Medical Officer LID, NIAID

Others: Suzanne U. Emerson, Ph.D. Microbiologist LID, NIAID
 Czeslaw Wychowski, Ph.D. Visit. Assoc. LID, NIAID
 Edward Cox Guest Researcher LID, NIAID
 Patricia Shields Guest Researcher LID, NIAID
 Robert Purcell, M.D. Head, HV Section LID, NIAID

COOPERATING UNITS (if any)

Johns Hopkins Medical School (Karron); Harvard Medical School (Cohen); LIG,
 NIAID (Maloy); Research Institute of Scripps Clinic, LaJolla, CA (Lerner); Univ. of N.C. (Lemon)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

1.2

PROFESSIONAL

0.7

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Antibodies to hepatitis A capsid proteins have been produced against purified, SDS-disrupted whole virions. In addition, Dr. Lee Maloy has prepared antibodies against a synthetic peptide representing the entire predicted 23 amino acids of VP4, and the carboxy terminus of VP2. Dr. Richard Lerner has prepared antibodies against a set of peptides spanning the entire predicted sequence of VP1. These antibodies were studied by immunoprecipitation and western blot analysis of the viral structural peptides. Because the predicted size for the capsid protein VP4 is only 23 amino acids (about 1/3rd the size of most picornavirus VP4's) we first questioned whether the cleavage of VP0 into VP2 and VP4 actually occurs. The antibody to VP4 specifically recognized a larger peptide in the 25 to 30 kDa range which is presumably VP0 (VP4 plus VP2). We could not identify a viral VP4 band in these western blots but the sensitivity of the test for synthetic VP4 was very low (approximately 1 µg of peptide). An antibody to a synthetic peptide representing the carboxy terminus of the capsid protein VP2 recognized 2 closely spaced bands (VP0 and VP2) on western blots. Thus, it appears that cleavage of VP0 to yield VP2 and VP4 does occur but it is possible that the very small VP4 is lost from the mature virion.

We have produced HAV "epitope libraries" in the expression vector lambda gt 11. A fragment of HAV cDNA representing the entire capsid coding region was isolated. Random fragments of this DNA were produced by limited DNase digestion and these fragments were cloned into lambda gt 11. These libraries were screened by neutralizing monoclonal antibodies, but specific clones were not identified. These results suggest that the epitopes recognized by these antibodies are discontinuous or conformational and a different approach will be required to identify them.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00311-07 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Search For New Hepatitis Agents		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Robert H. Purcell, M.D.	Head, HV Section LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Section LID, NIAID
COOPERATING UNITS (if any) Nat'l. Inst. Virology, Pune, India (K. Pavri); Medical College, Srinagar, India (M. S. Khuroo); Mt. Sinai Hosp., NY (Popper); USUHS, Bethesda, MD (Legters).		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Hepatitis Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	1.7	PROFESSIONAL: 0.3 OTHER: 1.4
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) In 1980 we described a new form of viral hepatitis caused by a previously unrecognized hepatitis virus with epidemiologic characteristics of HAV (i.e., the virus is fecal-orally transmitted, produces water-borne epidemics and is probably responsible for a significant proportion of endemic hepatitis) but not related to HAV. The disease has been called epidemic or enterically transmitted non-A, non-B hepatitis (ENANB). Such hepatitis is both epidemic and endemic throughout the Indian subcontinent, central Asia, parts of the Middle East and northern Africa, with probable extension to West Africa. The putative etiologic agent, a 27-30nm nonenveloped virus, was identified in a volunteer study in 1983 and similar virus-like particles have been detected in feces of cases in a number of other outbreaks. The small quantity of such virus-like particles available for study has limited progress. The objectives of this study are to identify and characterize the etiologic agent of ENANB hepatitis and to develop useful assays for diagnosis of infection and seroepidemiologic studies. A longer term objective is the development of passive and active immunoprophylaxis for this important human pathogen. The identification of an acute phase stool sample that contains marginally sufficient virus-like particles for use as an antigen in immune electronmicroscopic studies of ENANB has permitted limited cross-serologic studies of paired sera obtained from epidemic and endemic disease in regions where the virus is prevalent. From these studies we conclude that ENANB hepatitis occurring throughout India, in Pakistan, southern Russia and Algeria is all caused by serologically related or identical viruses. Chimpanzees inoculated with stools containing virus-like particles from the outbreaks developed hepatitis and seroconverted, thus proving transmissibility to a non-human primate species.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00312-06 LID
TERMINATED

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical and Experimental Studies of Hepatitis B Vaccines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Robert H. Purcell, M.D. Head, HV Sect. LID, NIAID

Others: John Ticehurst, M.D. Medical Officer LID, NIAID

COOPERATING UNITS (if any)

Baylor School Med., Houston, TX (Hollinger); Div. Mol. Virol. & Immunol., G.U., Wash. DC (Gerin); Dept. Med., Rutgers Med. School, New Brunswick, NJ (McAuliffe); CDC, Atlanta, GA (Francis); Dept. Epid., Shanghai 1st Med. Coll. (Xu).

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

0

PROFESSIONAL

0

OTHER

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00314-07 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Woodchuck Virus		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Roger Miller, Ph.D	Senior Staff Fellow LID, NIAID
Others:	Cathy Chung, Ph.D.	NRC LID, NIAID
	Rosina Girones, Ph.D.	Visit. Fellow LID, NIAID
	Shuichi Kaneko, M.D.	Visit. Fellow LID, NIAID
	Robert H. Purcell, M.D.	Head, HVS LID, NIAID
COOPERATING UNITS (if any) Div. Molec. Virol. & Immunol., Georgetown U., Wash., D.C. (J. Gerin); New York State College of Veterinary Medicine (B. Tennant).		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Hepatitis Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
4.8	3.8	1.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The woodchuck hepatitis virus (WHV) is taxonomically and serologically related to the hepatitis B virus (HBV). Infection with each of these viruses is associated with acute and chronic hepatitis and hepatic cell carcinoma in their respective hosts; these associations appear to be etiological in nature. Thus, the WHV/woodchuck system provides a relevant and convenient model for understanding HBV infections of humans. The following experiments were successfully completed: (1) Tests specific for the WHV antigen-antibody systems were developed. (2) Recombinant WHV DNA was transfected into the liver of susceptible woodchucks and acute and chronic WHV infections produced. (3) Genetically altered WHV genomes, with mutations in the virus X gene sequence, were constructed by recombinant DNA techniques. Mutant virus genomes were non-infectious in woodchucks, which implied that these X gene sequences were essential for virus replication. (4) Supercoiled WHV, from the nucleus of infected liver cells, was successfully cloned and the complete nucleotide sequence determined. The sequence of this replicative intermediate is identical to that of the partially double stranded WHV genome found in virions. (5) The complete nucleotide sequence of three additional WHV genomes was determined. It appears that virus subtypes of WHV are evolving, analogous to the surface antigen subtypes of HBV. (6) The in vivo fidelity of the WHV polymerase was determined. The mutation rate for WHV replication was found to be $<2 \times 10^{-4}$ changes per genome per year, which is lower than the mutation rate of retroviruses and other single strand RNA viruses. (7) Two transcripts specific for the X gene region of the WHV genome were discovered. One transcript, 0.65 kb in length, was localized to the nucleus of infected liver cells, and found to lack a poly(A) tail at its 3' end. The second transcript, 0.80 kb in length, was shown to be the poly-adenylated version of the 0.65 kb transcript.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00316-06 LID
TERMINATED

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (60 characters or less. Title must fit on one line between the borders.)

The Delta Agent

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Robert H. Purcell, M.D.

Head, HV Section

LID, NIAID

COOPERATING UNITS (if any)

Georgetown U., Washington, DC (Gerin); CDC, Phoenix, Ariz. (Hadler);
Sinai Hospital, NYC (Popper); Chiba U., Chiba City, Japan (Omata); U. Washington Med. Research
Unit, Taipei, Taiwan (Beasley)

LABORATORY

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

0

PROFESSIONAL

0

OTHER

0

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00317-07 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biology of Non-A, Non-B Hepatitis (NANBH) Agents		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Stephen M. Feinstone, M.D. Medical Officer	LID, NIAID
Others:	Robert H. Purcell, M.D. Head, HV Section	LID, NIAID
COOPERATING UNITS (if any) Blood Bank, NIH Clinical Center, Bethesda, MD (Alter, Shih, Esteban); CH, LMG, NIH, Bethesda, MD (Dawid, Sargent). Nihon U. School Med., Tokyo, Japan (Y. Shimizu); Cetus Corp., Emeryville, CA (Sninsky)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Hepatitis Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	0.8	0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Michael Houghton and colleagues at Chiron Corporation have cloned, expressed and sequenced at least part of the genome of the NANBH agent. They believe it is a 10KB, positive sense polyadenylated RNA virus that may be related to the alpha togaviruses. Sequence homology has not been found between the NANBH partial sequence and any other known viral sequence. I plan to use a variety of cDNA cloning procedures in order to confirm the Chiron findings. However, since they started with liter quantities of plasma that contained 10^6 infectious viruses/ml and I have approximately 100 ml of plasma of the same titer, the task is likely to be very difficult. It is hoped that the polymerase chain reaction technique will aid this effort. Molecular approaches to identifying the non-A, non-B hepatitis agent now involve development of technology that can be used to clone extremely small quantities of nucleic acid. We know that the NANBH agent is present in serum and even liver at very low levels, i.e., less than 10^6 infectious units per ml of serum or gram of liver. It also seems that if poly(A)+ NANBH mRNA is produced, it is a very low abundance message. Therefore, either direct cloning of DNA or cDNA cloning is technically very difficult. In order to assist cloning small quantities of DNA or cDNA, a "nonclonable" piece of RNA or DNA has been produced. This non-clonable fragment will be added to NANBH nucleic acid preparations to act as a carrier for all cloning steps. This non-clonable fragment will be useful for a number of different cloning strategies where the level of the nucleic acid of interest is very low.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES • PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00321-06 LID
TERMINATED

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (50 characters or less. Title must fit on one line between the borders.)

Development, Characterization, and Use of Cloned Hepatitis A Virus (HAV) cDNA

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: John Ticehurst, M.D. Medical Officer LID, NIAID

Others: Stephen M. Feinstone, M.D. Medical Officer LID, NIAID
Robert H. Purcell, M.D. Head, HV Sect. LID, NIAID
Jeffrey I. Cohen, M.D. Medical Staff Fellow LID, NIAID
Manfred Weitz, Ph.D. Fogarty Visit. Fellow LID, NIAID
Ruth A. Karron, M.D. Guest Worker LID, NIAID

COOPERATING UNITS (if any) G'town U. (Baroudy); Columb U. (Racaniello); U. Neb. (Tracy); U Wisc (Palmenberg); U Turku, Finland (Hyypia); Genentech Corp, CA (Kleid); Scripps, CA (Lerner); LMB, NCI (Maizel); LIG, NIAID (Maloy); Athens Sch. Hyg., Greece (Tassopoulos); Armed Forces Inst Pathol (Anderson); LVD, NIAID (Moss, Fuerst)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

0

PROFESSIONAL

0

OTHER

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00370-06 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of Acquired Immune Deficiency Syndrome (AIDS)		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Robert H. Purcell, M.D.	Head, HV Section LID, NIAID
Others:	Andrea Barnes, D.V.M.	Veterinary Medical Officer LID, NIAID
COOPERATING UNITS (if any) NINCDS, NIH, Bethesda, MD (London); NCI (Gallo); LIG, NIAID, NIH, Bethesda, MD (Folks, Fauci); Georgetown U. (Gerin, Hirsch, Johnson, Olmsted).		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Hepatitis Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.7	1.7	2.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) A new effort concentrating on animal models of AIDS was undertaken in August, 1987. Two model systems are being established: 1) a primate model using the simian immunodeficiency virus (SIV), and, 2) a feline model using the feline T-lymphotrophic virus (FTLV). Both of these viruses belong to the lentivirus subfamily and induce chronic persistent infection and immunodeficiency in their respective hosts. SIV is genetically closely related to the human AIDS viruses, while FTLV appears to be much less related to the human or primate viruses based on a lack of antigenic cross reactivity (sequence data is not available). Thus, these two model systems will allow an in-depth examination (<u>in vitro</u> and <u>in vivo</u>) of the molecular biology, pathogenesis, and host immune responses, and will afford the opportunity to evaluate systematically candidate vaccine approaches to immunoprophylaxis and antiviral therapies.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00404-04 LID
TERMINATED

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (30 characters or less. Title must fit on one line between the borders.)

Second and Third-Generation Hepatitis B Vaccines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Robert H. Purcell, M.D. Head, HV Section LID, NIAID

COOPERATING UNITS (if any)

Georgetown University, Washington, DC (Gerin); Scripps Institute, La Jolla, CA (Lerner); LBV, NIAID, NIH, Bethesda, MD (Moss)

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Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

0

PROFESSIONAL

0

OTHER

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00405-04 LID
TERMINATED

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Inactivation of Hepatitis Viruses in Pooled Plasma Derivatives

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Robert H. Purcell, M.D.

Head, HV Section

LID, NIAID

COOPERATING UNITS (if any)

Georgetown U., Washington DC (Gerin); Southwest Fndt., San Antonio, TX
(Eichberg); Revlon Inc., Tuckahoe, NY (Landaburu)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00406-05 LID

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Structure of HAV: Genomic Sequence and Organization

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator: (Name, title, laboratory, and institute affiliation))

PI:	Suzanne U. Emerson, Ph.D.	Microbiologist	LID, NIAID
Others:	Czeslaw Wychowski, Ph.D.	Visit. Assoc.	LID, NIAID
	Stephen M. Feinstone, Ph.D.	Medical Off.	LID, NIAID
	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

1.4

PROFESSIONAL

0.9

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|---|
| ☐ (a) Human subjects | ☐ (b) Human tissues | ☒ (c) Neither |
| ☐ (a1) Minors | | |
| ☐ (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

RNAs transcribed from the cDNA clones of wild-type, cell culture adapted hepatitis A virus, or mutants constructed from these clones are being translated in vitro to define the parameters affecting the efficiency of translation. Deletion mutants have been constructed to answer the question of whether hepatitis A viability requires an intact VP4 gene or whether myristylation of VP4 is important.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00440-04 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Construction of Full Length Hepatitis A Virus cDNA For Transfection		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	Suzanne Emerson, Ph.D.	Microbiologist LID, NIAID
Others:	Stephen M. Feinstone, M.D. Robert H. Purcell, M.D.	Medical Officer LID, NIAID Head, HV Section LID, NIAID
COOPERATING UNITS (if any) Columbia University (V. Racaniello); WRAIR (J.R. Ticehurst)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Hepatitis Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.6	0.4
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) Chimeric cDNA which contain portions of both wild-type and attenuated HAV cDNA were constructed. RNA transcripts from several of these cDNA constructs were infectious when transfected into monkey kidney cells and the results indicated that two genes, 2B and 2C, were important for tissue culture adaptation. Site-directed mutagenesis and transfection assays suggested that at most, four of six base changes in the attenuated clone are sufficient to allow wild-type virus to grow in cell culture. These mutations will be inserted by site-directed mutagenesis into the wild-type clone and transfection assays will be done to determine the minimum number of mutations which allow growth in tissue culture. Viruses from the transfection experiments will be inoculated into marmosets to determine which portions of the HAV genome are associated with attenuation and how this phenotype relates to the ability to grow in cell culture. The 2B and 2C genes of the cell adapted HAV will be used to permanently transform a continuous monkey cell line in an attempt to generate a complementing cell line which supports the growth of wild-type virus or improves the growth of the cell adapted virus.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00473-02 LID
TERMINATED

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Replication of Hepatitis A Virus In Vivo

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Jeffrey I. Cohen, M.D. Medical Staff Fellow LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

0

PROFESSIONAL

0

OTHER

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00530-01 LID
PERIOD COVERED <u>October 1, 1987 to September 30, 1988</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Molecular and computer analysis of the hepatitis B virus genome</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Roger H. Miller, Ph.D.	Senior Staff Fellow LID, NIAID
Others:	Cathy Chung, Ph.D. Rosina Girones, Ph.D. Shuichi Kaneko, M.D. Robert H. Purcell, M.D.	NRC Visit. Fellow Visit. Fellow Head, HVS
		LID, NIAID LID, NIAID LID, NIAID LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH <u>Laboratory of Infectious Diseases</u>		
SECTION <u>Hepatitis Viruses Section</u>		
INSTITUTE AND LOCATION <u>NIAID, NIH, Bethesda, MD 20892.</u>		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
<u>.2</u>	<u>.2</u>	<u>0</u>
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
The purpose of this project is to study the HBV genome by computer and molecular hybridization analysis. Our data suggest that HBV and retroviruses evolved from a common ancestor. This is a significant finding because it demonstrates the relatedness of two diverse virus families. We find that: (1) both HBV and retroviruses utilize a unique mechanism of genome replication by reverse transcription of RNA; (2) highly conserved regions of HBV proteins share considerable homology with retrovirus nucleocapsid, protease, and polymerase proteins; (3) the HBV polymerase shares organizational similarities with the polymerase gene sequence of retroviruses and retrovirus-like genetic elements; (4) genetic analysis and low stringency blot hybridization indicates that HBV DNA sequences are most closely related to MLV DNA sequences; (5) HBV possesses two previously undiscovered open reading frames which may represent genuine gene sequences. Interestingly, one of the new open reading frames is located on the DNA plus strand of the double-stranded virus genome. This also appears to be a unique feature of retroviruses that encode proteins involved in transcription activation because we find a plus strand DNA ORF in the human immunodeficiency virus genome, and in the genomes of related retroviruses of humans and animals. Overall, HBV and retroviruses share a common mechanism of genome replication, possess homologous nucleotide and amino acid, and have genomes that share a similar organization of genes. Therefore, it is probable that these virus families evolved from a common ancestral virus.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00323-07 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structure and Expression of Parainfluenza Type 3 Virus Genes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)		
PI:	Peter L. Collins, Ph.D.	Senior Staff Fellow LID, NIAID
Others:	Melanie K. Spriggs, Ph.D. Brian R. Murphy, M.D.	Staff Fellow LID, NIAID Head, RV Section LID, NIAID
COOPERATING UNITS (if any) NINCDS, NIH (Dr. William T. London)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Respiratory Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.75	1.25	0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Previously, we constructed recombinant vaccinia viruses that expressed individually the hemagglutinin-neuraminidase (HN) and fusion (F) glycoproteins of human parainfluenza virus type 3 (PIV3). Intradermal immunization of rodents and non-human primates induced high levels of protective immunity and demonstrated the feasibility of a subunit vaccine for PIV3. In continuation of this work, we are constructing analogous recombinant vaccinia viruses using parental strains (Wyeth and Lister) that are suitable for evaluating immunogenicity in humans. Also, recombinant adenoviruses that will express individually the HN and F proteins are under construction. The intracellular synthesis and maturation of the HN protein was investigated by the expression of cDNAs that had been engineered to encode N-terminally truncated HN proteins. The truncated HN proteins contained progressively larger deletions of the N-terminal cytoplasmic and transmembrane domains. The results indicated that the folding of the HN ectodomain into a biologically active and conformationally authentic form occurs to completion in the rough endoplasmic reticulum. However, the finding that two biologically-active HN mutants remained localized primarily in the rough endoplasmic reticulum indicated that correct folding was not the sole prerequisite for movement through the exocytotic pathway. Finally, the glycosylation of the HN protein (which contains only N-linked sugars) and the respiratory syncytial virus G protein (which contains both N-linked and O-linked sugars) was compared. By cDNA mutagenesis and expression, chimeric proteins were expressed that consisted of the N-terminal cytoplasmic and membrane domains of each protein fused to the ectodomain of the other. This study showed that the processing and maturation of each ectodomain was independent of the origin of the cytoplasmic and membrane domains.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00324-07 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Laboratory Studies of Myxoviruses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Mark H. Snyder, M.D.	Senior Staff Fellow LID, NIAID
Others:	John T. Treanor Brian R. Murphy, M.D. Robert M. Chanock, M.D.	Senior Staff Fellow Head, RV Section Chief LID, NIAID LID, NIAID LID, NIAID
COOPERATING UNITS (if any) U. of Michigan (Maassab, DeBorde)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Respiratory Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 3.35	PROFESSIONAL: 1.7	OTHER: 1.65
CHECK APPROPRIATE BOXES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) A cold-adapted (ca) reassortant influenza B virus vaccine was restricted in replication in the upper and lower respiratory tract of hamsters and chimpanzees compared to its wild type influenza B virus parent. Isolates of the influenza B ca reassortant recovered after up to 15 days of replication in immunosuppressed hamsters did not exhibit instability of the ts or attenuation phenotype as indicated by tests performed in cell culture and in hamsters. Attempts are underway to isolate phenotypic revertants of previously characterized influenza A virus reassortants which derived only one gene from the influenza A/Ann Arbor/60 ca donor virus, while the remaining genes were derived from a wild type influenza A virus. Each of four ts⁺ revertants of a single gene PB2 reassortant virus also regained the ability to replicate in the lower respiratory tract of hamsters indicating that attenuation specified by the ca PB2 gene is closely linked to the ts phenotype. Phenotypic revertants of single gene PB1 and M reassortant viruses have also been isolated and are currently under evaluation. The NS gene of avian influenza A viruses exhibits sequence dimorphism, i.e., there are two allelic forms of the NS gene, allele A or allele B. One of these alleles (A) is also present in human influenza A viruses, while the other avian influenza A virus NS allele (B) is highly divergent from the corresponding gene of the human influenza A viruses. The level of replication of these reassortant viruses in squirrel monkeys will be compared with that of a human wild type influenza A virus to determine if the B allele specifies host range restriction in primate respiratory tissue. Phenotypic and genotypic stability are important properties of the M and NP genes of the avian influenza A/Mallard/78 donor virus that play a role in host-range restriction of avian-human influenza A virus vaccines in primates. Both phenotypic and genotypic stability of these genes are being studied by biological and sequence analysis of single M or NP gene reassortants which have been passaged serially in monkeys.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00325-07 LID																				
PERIOD COVERED October 1, 1987 to September 30, 1988																						
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Study of Respiratory Viruses in Primates																						
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">Brian R. Murphy, M.D.</td> <td style="width: 30%;">Head, RV Section</td> <td style="width: 20%;">LID, NIAID</td> </tr> <tr> <td>Others:</td> <td>Mark H. Synder, M.D.</td> <td>Senior Staff Fellow</td> <td>LID, NIAID</td> </tr> <tr> <td></td> <td>Robert M. Chanock, M.D.</td> <td>Chief</td> <td>LID, NIAID</td> </tr> <tr> <td></td> <td>Robert Olmsted, Ph.D.</td> <td>Staff Fellow</td> <td>LID, NIAID</td> </tr> <tr> <td></td> <td>Peter L. Collins, Ph.D.</td> <td>Senior Staff Fellow</td> <td>LID, NIAID</td> </tr> </table>			PI:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID	Others:	Mark H. Synder, M.D.	Senior Staff Fellow	LID, NIAID		Robert M. Chanock, M.D.	Chief	LID, NIAID		Robert Olmsted, Ph.D.	Staff Fellow	LID, NIAID		Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID
PI:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID																			
Others:	Mark H. Synder, M.D.	Senior Staff Fellow	LID, NIAID																			
	Robert M. Chanock, M.D.	Chief	LID, NIAID																			
	Robert Olmsted, Ph.D.	Staff Fellow	LID, NIAID																			
	Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID																			
COOPERATING UNITS (if any) Meloy Laboratories, Rockv., MD (J. Philips); NINCDS, NIH, (W.T. London); LVD, NIAID, NIH (B. Moss, R.M. Buller); Johns Hopkins U. Schl. Pub. Health, Balt., MD (G. Prince); FDA (M. Hendry)																						
LAB/BRANCH Laboratory of Infectious Diseases																						
SECTION Respiratory Viruses Section																						
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892																						
TOTAL MAN-YEARS	PROFESSIONAL	OTHER																				
1.1	0.2	0.9																				
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews																						
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) A cold-adapted (ca) influenza B reassortant virus constructed from the ca donor virus of Maassab was attenuated in the upper and lower respiratory tract of chimpanzees. Each of the isolates from these primates retained the temperature sensitivity phenotype after replication in the chimpanzee. These observations indicated that influenza B ca reassortants were attenuated and stable genetically in chimpanzees and should be studied further in human volunteers. Infection by vaccinia-respiratory syncytial virus (RSV) glycoprotein recombinants induced a high level of resistance to RSV challenge in the upper and lower respiratory tract of owl monkeys. This indicated that intradermal infection by a vaccinia-RSV recombinant can provide a high level of resistance to a virus that replicates on respiratory tract mucosa and offers encouragement for the ultimate success of vaccinia-RSV recombinants as vaccines for humans. A vaccinia TK⁺ recombinant virus was markedly attenuated in dermal virulence for patas monkeys when the lymphokine IL-2 was co-expressed. A TK⁻ vaccinia recombinant that co-expressed IL-2 was also less virulent than a TK⁻ recombinant that did not co-express IL-2. Significantly this attenuation effected by IL-2 did not reduce immunogenicity appreciably. SIV and STL^V could not be detected in suspensions of parainfluenza virus type 3 and various rotaviruses that had a history of passage in monkey kidney tissue culture and are now being considered for use in humans. Absence of contamination of the candidate vaccine viruses are free of contamination by these two horizontally transmitted retroviruses it is reasonable to continue our evaluation of the candidate vaccines in human volunteers.																						

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00326-07 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Study of Respiratory Viruses in Volunteers		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Mark H. Snyder, M.D.	Senior Staff Fellow LID, NIAID
Others:	Brian R. Murphy, M.D. Robert M. Chanock, M.D.	Head, RV Section Chief LID, NIAID LID, NIAID
COOPERATING UNITS (if any) Flow Labs, Rockv. MD (Potash); Johns Hop. U. Balt. MD (Clements, Sears, Steinhoff, Halsey); U. Roch. S. Med. Roch. NY (Betts, Dolin); Vanderbilt U.S. Med. Nashv. TN (Wright); Marshall U.S. Med., Huntington, WVA (Belshe)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Respiratory Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	0.7	1.4
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
The avian-human (AH) influenza A/Mallard/NY/6750/78 and <u>ca</u> A/Ann Arbor/6/60 reassortant viruses were compared for safety, attenuation, infectivity, immunogenicity, and efficacy against attenuated virus challenge in tests performed in fully susceptible infants and children, 6-48 months of age. The reassortant viruses were derived from the wild type human influenza A/Bethesda/1/85 (H3N2) or influenza A/Kawasaki/9/86 (H1N1) virus. The <u>ca</u> and AH reassortants derived from the same wild virus type donor were similar with respect to attenuation, immunogenicity, infectivity and efficacy. However, both AH and <u>ca</u> H1N1 reassortants appeared to be more infectious than their H3N2 counterparts. A single infection induced complete resistance to subsequent challenge with the homologous attenuated virus. However, 55-60% of vaccinees developed a significant antibody response to a second dose of vaccine. Reassortants prepared from two avian influenza A virus donors, A/Mallard/NY/6750/78 and A/Mallard/Alberta/88/76, and the same human influenza A/Bethesda/85 (H3N2) wild type virus were compared in seronegative adult volunteers. The reassortants appeared to be similar, hence, future efforts will be directed toward the further evaluation of reassortants derived from the better characterized A/Mallard/NY/6750/78 donor virus. A dose response study was performed to evaluate the attenuation, immunogenicity, infectivity and efficacy of <u>ca</u> reassortant influenza B/Ann Arbor/1/86 virus in seronegative young adults. The level of attenuation, immunogenicity, and efficacy were satisfactory. In immunogenicity tests performed in elderly persons (65-83) simultaneous administration of live influenza A vaccine and inactivated influenza virus vaccine was superior to either vaccine alone. The ELISA test for detecting immune responses to virus infection was modified to utilize new spectrophotometers which can measure the rate of color development in microtiter wells. This new kinetic based ELISA requires only a single dilution of specimen and yields results which are comparable in terms of sensitivity and specificity to those produced by older endpoint ELISA's which require multiple dilutions.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00327-07 LID

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Parainfluenza Type 3 Virus Surface Glycoproteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Kathleen Coelingh, Ph.D. Senior Staff Fellow LID, NIAID

COOPERATING UNITS (if any)

Texas A&M University (M. Summers); LCI, NIAID (S. Vasantha, L. Fries)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

1.75

PROFESSIONAL

0.75

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have produced baculovirus recombinants expressing the human parainfluenza type 3 virus (PIV3) hemagglutinin-neuraminidase (HN) protein to extend our previous antigenic and functional characterization of the HN to include structural analysis. The HN protein expressed by initial baculovirus recombinants was shown to be glycosylated, biologically active, immunogenic, and antigenically authentic, but was produced in relatively small quantities. We have now produced a second generation of recombinants which express HN as a fusion product. These new recombinants achieve a level of expression which is 20-fold higher than our initial recombinants. We have also generated recombinants which produce a truncated, secreted form of HN. Recombinant HN protein will be purified prior to structural analysis.

To extend our antigenic and functional analysis of the PIV3 surface glycoproteins, we have produced monoclonal antibodies (MAbs) to the fusion (F) glycoprotein. These MAbs define 20 epitopes, 14 of which participate in neutralization. The neutralization epitopes are distributed among three non-overlapping antigenic sites (A,B, and C) and one bridge site (AB), and the six non-neutralization epitopes form four distinct antigenic sites. Analysis of the biological activities of MAbs indicated that antigenic sites AB,B, and C correspond to functional domains of the F protein. Antigenic variants have been selected using neutralizing and fusion-inhibiting MAbs, and sequence analysis of these variants and of naturally-occurring clinical PIV3 isolates is currently in progress. These studies will identify amino acid residues involved in neutralization by antibodies as well as residues important for fusion function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00344-06 LID TERMINATED
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunologic Factors in Respiratory Syncytial Virus (RSV) Disease		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	Brian R. Murphy, M.D.	Head, RVS LID, NIAID
Others:	Robert M. Chanock, M.D.	Chief LID, NIAID
	Gregory A. Prince, D.D.S., Ph.D.	Guest Worker LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Respiratory Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	0	PROFESSIONAL: 0 OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<u>TERMINATED</u>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00345-07 LID

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunity to RS Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Brian R. Murphy, M.D.	Head, RVS	LID, NIAID
Others:	Robert M. Chanock, M.D.	Chief	LID, NIAID
	Gregory Prince, D.D.S., Ph.D.	NIH Spec. Vol.	LID, NIAID
	Peter Collins, Ph.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS (if any)

USUHS, Bethesda, MD (V. Hemming); Praxis Biologics, Rochester N.Y. (P. Paradiso), Georgetown University (Jensen).

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

2.1

PROFESSIONAL

0.2

OTHER

1.9

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Immunization of cotton rats with vaccinia RSV F and G recombinants induced resistance to RSV challenge without potentiating disease even in animals previously administered varying amounts of RSV antiserum. Passive transfer of respiratory syncytial virus (RSV) antiserum suppressed the immune response to the RSV fusion (F) and large (G) glycoproteins expressed by recombinant vaccinia viruses administered intradermally. The suppressive effect of passively administered RSV immune serum could be largely overcome by administering recombinant vaccinia virus intranasally or by inoculating a large quantity of purified F and G glycoproteins intramuscularly.

Immunization of cotton rats with highly purified fusion (F) and large (G) glycoproteins of respiratory syncytial virus induced resistance to RSV challenge without potentiating disease. This suggests that purified glycoproteins can be used safely in a vaccine against RSV. Although, the F and G glycoprotein vaccine induced a high titer of F and G specific antibodies these antibodies had low neutralizing activity.

Systemic or topical administration of RSV hyperimmune antiserum to cotton rats hastened recovery from infection. It was found that ADCC and complement were not required for the full therapeutic effect of the passively transferred RSV antibody which suggests that virus neutralization is the major mechanism by which the antiviral effect is achieved.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00368-06 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structural Analysis of Respiratory Syncytial Virus Genome		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	Peter L. Collins, Ph.D.	Senior Staff Fellow LID, NIAID
Others:	Melanie K. Spriggs, Ph.D. David S. Stec, Ph.D.	Senior Staff Fellow LID, NIAID Staff Fellow LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Respiratory Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.75	1.25	0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
In previous work (annual reports, 1984-1987), we cloned and sequenced nine of the ten genes of human respiratory syncytial virus strain A2, which represents RSV antigenic subgroup A. Also, cloning and sequencing was extended to the Long strain, which is a second strain from antigenic subgroup A, and the 18537 strain, which represents subgroup B (annual reports, 1986 and 1987). To continue the structural analysis of RSV genomic RNA (vRNA) and its encoded mRNAs and proteins, cDNA cloning and sequencing is underway for the L gene of strain A2. Other work in progress is designed to determine the sequences of the 5' and 3' termini of vRNA of strain A2 by direct RNA sequencing and by cDNA cloning and sequencing. This will complete the sequencing of vRNA of the prototype A2 strain. The availability of cDNAs and sequences representing the entire vRNA will be an important step towards the goal of expressing infectious RSV from cloned cDNA.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00372-06 LID

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

RSV Proteins: Roles in Host Immunity and Immunoprophylaxis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)

PI:	Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Robert A. Olmsted, Ph.D.	Staff Fellow	LID, NIAID
	Melanie K. Spriggs, Ph.D.	Staff Fellow	LID, NIAID
	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS (if any)

LVD, NIAID, NIH (Moss, Buller); Johns Hopkins U. Sch. of Pub. Hlth, Balt., MD (Prince); Wyeth Laboratory, Rancor, PA (Davis, Hung); MicroGeneSys Inc., West Haven, CT (Cochran, Smith).

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

0.75

PROFESSIONAL

0.25

OTHER

0.50

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

We are investigating approaches for utilizing recombinant DNA techniques in developing a vaccine for human respiratory syncytial virus (RSV). Previously, vaccinia virus recombinants that express individually the RSV F and G glycoproteins were shown to be highly immunogenic and effective in inducing resistance to challenge RSV infection in rodents and non-human primates. Analogous recombinants are being constructed with vaccinia virus strains (Wyeth and Lister) that are acceptable for use in humans. Vaccinia virus recombinants also have been constructed with seven other RSV genes, namely the 1C, 1B, N, P, M, 22K and 1A genes. Expression of the authentic RSV protein has been confirmed in each case, and these recombinants are being used to evaluate possible roles for these proteins in host immunity and virus-induced pathology. Two different adenovirus type 5 recombinants that contain the RSV F gene under the control of adenovirus promoters have been constructed, and these are being analyzed to determine whether the F protein is synthesized and processed authentically in recombinant-infected cells. Recombinant baculoviruses that express individually the F and G proteins are being evaluated as a potential source of purified F and G protein for a non-replicating subunit vaccine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00457-04 LID

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Human Immune Response to Respiratory Syncytial Virus Infection or Vaccination

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Brian R. Murphy, M.D. Head, RV Section LID, NIAID

Others: Gregory Prince, D.D.S., Ph.D. Spec. Volunteer LID, NIAID
Robert M. Chanock, M.D. Chief LID, NIAID

COOPERATING UNITS (if any)

U. Roch. Sch. Med. Roch., N.Y. (Walsh); Ch. Hosp. Nat. Med. Ctr. D.C. (Kim, Parrott); U.N. Carolina, Chapel Hill, N.C. (Henderson)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

0.6

PROFESSIONAL:

0.2

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The response of infants and children to first infection with RSV subgroup A or subgroup B respiratory syncytial virus (RSV) was characterized using an ELISA specific for each G glycoprotein. Infection with a subgroup A virus gave a broad response to the G glycoprotein of both A and B viruses, whereas the response to subgroup B virus was predominantly homotypic. Subgroup specific neutralization assays are currently being performed on sera obtained following first and second infections with RSV.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00474-02 LID
TERMINATED

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Molecular Mechanisms Underlying Adenovirus Pathogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Harold S. Ginsberg, M.D.	IPA	LID, NIAID
Others:	Robert M. Chanock, M.D.	Chief	LID, NIAID
	Gregory Prince, D.D.S., Ph.D.	Guest Worker	LID, NIAID
	Jorge Flores, M.D.	Visit. Scientist	LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Respiratory Virus Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects	☐ (b) Human tissues	☒ (c) Neither
☐ (a1) Minors		
☐ (a2) Interviews		

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00497-02 LID
PERIOD COVERED <u>October 1, 1987 to September 30, 1988</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Characterization of the Fusion Glycoprotein of Respiratory Syncytial Virus</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	Kathleen Coelingh, Ph.D.	Senior Staff Fellow LID, NIAID
Others:	Judith A. Beeler, M.D.	Fellow LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH <u>Laboratory of Infectious Diseases</u>		
SECTION <u>Respiratory Viruses Section</u>		
INSTITUTE AND LOCATION <u>NIAID, NIH, Bethesda, MD 20892</u>		
TOTAL MAN-YEARS: 1.75	PROFESSIONAL: 1.25	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) We have used 18 neutralizing monoclonal antibodies (MAbs) specific for the respiratory syncytial virus (RSV) fusion (F) glycoprotein to construct operational, topologic, and functional epitope maps of the F glycoprotein. The MAbs define 16 neutralization epitopes which are organized into three non-overlapping antigenic sites (A,B,and C) and one bridge site (AB). Neutralization and fusion-inhibition tests of the MAbs indicated that antigenic sites A,AB,and C correspond to functional domains of the F protein involved in syncytium-formation, whereas antigenic site B does not participate directly in the fusion process. This conclusion is reinforced by two additional observations. First, MAb-resistant variants selected with site B MAbs produce normal plaques, whereas those selected with MAbs binding epitopes in sites A and AB produce pinpoint plaques. Second, epitopes in antigenic sites A,AB,and C (which correspond to functional domains) were less variable among RSV clinical isolates than were epitopes in antigenic site B. Sequence analysis of the F genes of antibody-resistant mutants will allow us to identify amino acid residues which are responsible for binding neutralizing antibodies and which participate in syncytium-formation.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00498-02 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Synthesis, Processing and Functions of the Proteins of Human RSV		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Peter L. Collins, Ph.D.	Senior Staff Fellow LID, NIAID
Others:	Robert A. Olmsted, Ph.D. Melanie K. Spriggs, Ph.D.	Staff Fellow LID, NIAID Staff Fellow LID, NIAID
COOPERATING UNITS (if any) Northwestern Univ., Evanston, IL (Lamb); LIG, NIAID, NIH (W. Lee Maloy)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Respiratory Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.75	1.25	0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The structure and processing of the small 1A protein of human respiratory syncytial virus (RSV) was investigated. The 1A protein accumulated intracellularly as four species: two species (4.8K and 7.2K) were unglycosylated and differed in length because translational initiation for the synthesis of the 4.8K species occurred at the second methionine in the sequence. The two other forms (13-15K and 21-30K) contained an N-linked carbohydrate side chain. The 13-15K species appeared to be converted into the 21-30K form by the post-translational addition of polylactosamine to the N-linked side chain. This complex processing pathway for 1A was conserved between the RSV antigenic subgroups. All forms of 1A were shown to be inserted into intracellular membranes regardless of glycosylation status, and all forms except 4.8K were transported to the cell surface. Protein-mapping studies showed that the C-terminus of the 1A protein is extracellular, whereas the N-terminus is cytoplasmic. Additional studies to investigate the structures and processing of other RSV proteins are discussed.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00458-02 LID

INACTIVE 1987/88

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Variations Among Dengue Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Yi-ming Zhang, M.D.

Visiting Fellow

LID, NIAID

Others: Ching-Juh Lai, Ph.D.

Head, MVB Section

LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

0

PROFESSIONAL

0

OTHER:

0

CHECK APPROPRIATE BOXES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The dengue virus subgroup of the flavivirus family is comprised of 4 distinct serotypes (type 1 to type 4). Intratypic variations have been detected within several serotypes by oligonucleotide fingerprint analysis and by virus neutralization. Dengue type 4 variants from the Caribbean recovered during 1981-1982 appear to be different from the dengue 4 prototype (H241) isolated in the Philippines in 1956 as well as recent isolates from Southeast Asia. During the past two years, dengue type 4 was the most frequent cause of dengue hemorrhagic shock in Bangkok, in contrast to the earlier epidemic pattern in which dengue type 2 viruses were most often implicated. For these reasons it is important to define in molecular terms: (a) genetic stability and diversity of dengue 4 viruses isolated over a 30 year interval and (b) the possible involvement of specific virus strains, i.e., "virulent viruses", in severe dengue disease. Two dengue type 4 variants, the prototype strain H241 and strain 2123 isolated from a hemorrhagic shock patient, were chosen for cloning and DNA sequence analysis. Several specific oligonucleotide primers corresponding to the established dengue 4 sequence (Dominica strain) were tested for their ability to prime reverse-transcription. At least one oligonucleotide effectively primed each dengue genomic RNA and yielded cDNA 5,000 nucleotides in length. These cDNA products will be cloned by the procedure established earlier. Cloned DNA will then be analyzed to determine the sequence coding for the two protective antigens, the envelope glycoprotein and the NS1 nonstructural protein. Finally, sequences will be compared in an attempt to identify hypervariable regions in the E and NS1 glycoproteins.

INACTIVE 1987/88

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00459-04 LID
PERIOD COVERED <u>October 1, 1987 to September 30, 1988</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Expression of Dengue Virus Proteins Using Baculovirus as a Vector</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Yi-ming Zhang, M.D. Others: Ching-Juh Lai, Ph.D. Robert Chanock, M.D.	Visit. Assoc. Head, MVB Section Chief	LID, NIAID LID, NIAID LID, NIAID
COOPERATING UNITS (if any) WRAIR, Washington, D.C. (Summers, Eckels, Dubois); Rochester General Hospital, Univer. of Roch., Rochester, N.Y. (J.J. Schlesinger)		
LAB/BRANCH <u>Laboratory of Infectious Diseases</u>		
SECTION <u>Molecular Viral Biology Section</u>		
INSTITUTE AND LOCATION <u>NIAID, NIH, Bethesda, MD 20892</u>		
TOTAL MAN-YEARS: 0.95	PROFESSIONAL: 0.75	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The use of genetically engineered dengue proteins represents a promising vaccine strategy. We have employed the high yielding baculovirus-insect cell system to achieve expression of dengue viral proteins from cloned DNA sequences. A recombinant baculovirus containing a 4.0 kilo-base dengue virus cDNA sequence that codes for the three viral structural proteins, capsid (c) protein, premembrane (PreM) protein, and envelope glycoprotein (E), and nonstructural proteins NS1 and NS2a. Cultured insect cells infected with this recombinant virus produced what appeared to be authentic E and NS1. Immunization of mice with these dengue protein products induced resistance to fatal dengue encephalitis. To further elucidate the basis for this protective immunity, recombinant baculovirus that expressed only E or NS1 was constructed. Mice immunized with an infected cell lysate containing either E or NS1 also developed complete resistance to dengue virus challenge.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00475-03 LID

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Complete Nucleotide Sequence of Dengue Type 4 Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)

PI: Ching-Juh Lai, Ph.D

Head, MVB

LID, NIAID

Others: Bangti Zhao, Ph.D
Lewis Markoff, M.D.Visit. Scient.
Medical Officer,LID, NIAID
LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

0.5

PROFESSIONAL:

0.3

OTHER

0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A cDNA library from the dengue type 4 virus 42s RNA (strain 814669) was constructed. Multiple overlapping DNA clones which represent the entire dengue genome were used to determine the complete nucleotide sequence. The genome contains 10,644 nucleotides with a single open reading frame that encodes a polyprotein of 3386 amino acids. Homology alignment of the dengue type virus polyprotein with the polyproteins of two other flaviviruses, yellow fever and West Nile viruses, indicated that the three structural proteins designated capsid (c), membrane (M), and envelope (E) are located at the N-terminus and nonstructural proteins in the order of NS₁, NS_{2a}, NS_{2b}, NS₃, NS_{4a}, NS_{4b}, NS₅ are located at the C-terminus. The E glycoprotein is responsible for viral functional activities such as absorption to host cells, hemagglutination and reactivity with neutralizing antibodies. Amino and homology analysis indicates that there is a close sequence relationship among the envelope glycoproteins of different flaviviruses. The dengue NS₁ nonstructural protein has been shown to be the soluble complement fixing antigen. Recent studies with YF or dengue virus have shown that NS₁ induces immunity in mice against its respective virus infection. The use of NS₁ nonstructural protein for immunization against dengue is especially attractive because the possibility of immune enhancement due to envelope glycoprotein-specific antibodies could be avoided.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00476-03 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Engineering the Genome of Dengue Virus		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Bangti Zhao, Ph.D.	Visit. Scientist	LID, NIAID
Others: Ching-Juh Lai, Ph.D. Robert M. Chanock, M.D.	Head, MVB Section Chief	LID, NIAID LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Molecular Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.15	PROFESSIONAL: 0.95	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.) Overlapping cDNA clones were initially identified which spanned the entire dengue 4 sequence of 10,644 nucleotides in length. These separately cloned DNA segments were used to construct a full-length DNA copy. SV40 sequences that contained an early promoter and origin of SV40 DNA replication were inserted into the dengue plasmid and the resulting DNA (SVD4) was used for transfection of COS-1 cells. We also constructed recombinant dengue DNA containing an SP6 promoter in order to produce RNA transcripts for assay of infectivity in transfected LLCMK₂ cells. Special emphasis has been given to removing non-viral sequences from both termini of full-length dengue DNA. The modified SP6-dengue 4 DNA produced precise transcripts in an <u>in vitro</u> system including a cap structure at the 5'-end. Thus far, none of these transcripts has proved to be infectious following the standard transfection assay. Direct transfection of COS-1 cells with SV-D4 DNA also failed to demonstrate infectivity. It is possible that our failure to detect infectivity with our cloned DNA resulted from deletion or substitution mutations present in the virus which was cloned or such alterations might have been introduced as an artifact during cloning. A second full-length dengue cDNA copy has been constructed from a new set of separate clones. Although the RNA transcripts made from the new clone also failed to show infectivity, these new full-length dengue sequences should prove to be valuable for identifying mutations in this and other full length clones by comparison of restriction enzyme sites and sequence among the various clones. In addition, fragments in both non-infectious clones will be replaced with the corresponding fragments derived from other cloned cDNAs in a continuing attempt to construct a full length cDNA that is infectious.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00477-03 L1D
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT 80 characters or less Title must fit on one line between the borders Expression of Dengue Structural Proteins and NS1 by a Recombinant Vaccinia Virus		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name title laboratory and institute affiliation)		
PI:	Bangti Zhao, Ph.D.	Visit. Scientist LID, NIAID
Others:	Michael Bray, M.D. Ching-Juh Lai, Ph.D.	Staff Fellow LID, NIAID Head, MVBS LID, NIAID
COOPERATING UNITS (if any) WRAIR, Washington, D.C. (Eckels)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Molecular Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD, 20892		
TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.7	0.5	0.2
CHECK APPROPRIATE BOXES)		
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither		
☐ (a1) Minors		
☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
We employed vaccinia virus as a vector to express the three structural proteins, capsid (c), membrane (M) or its precursor PreM, and envelope glycoprotein (E), as well as the nonstructural protein NS1 from a 5'-terminal fragment of cloned dengue virus DNA. We observed that the three dengue virus glycoproteins, PreM, E, and NS1 were produced in recombinant vaccinia virus-infected cells and their glycosylation pattern was similar to that observed during dengue virus infection. Infection of cotton rats with the vaccinia virus recombinant induced a poor immune response to the NS1 glycoprotein, while antibodies for the other two glycoproteins were not detected at all. Nevertheless, mice immunized with this vaccinia virus recombinant developed complete resistance to fatal dengue encephalitis.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00499-02 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (90 characters or less. Title must fit on one line between the borders.) Expression of selected dengue E protein sequences by recombinant vaccinia virus		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	Michael Bray, M.D. Ching-Juh Lai, Ph.D. Robert M. Chanock, M.D.	Staff Fellow Medical Officer Head, MVB Section Chief LID, NIAID LID, NIAID LID, NIAID
COOPERATING UNITS (if any) Laboratory of Viral Diseases, NIAID, (B. Moss)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Molecular Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS 1.15	PROFESSIONAL 0.95	OTHER 0.2
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) A vaccinia recombinant expressing dengue type 4 virus structural (C,M,E) and nonstructural (NS1, NS2a) proteins failed to induce a detectable immune response to the envelope (E) glycoprotein in cotton rats or rabbits. Because of the possibility that cell surface expression might increase immunogenicity, we employed a vaccinia virus vector to express the dengue E protein on the cell surface, using the RSV G glycoprotein as a carrier. Recombinant DNA constructs were prepared that encoded a truncated N-terminal segment of the RSV G glycoprotein fused to an 85% fragment of the (E) glycoprotein or to smaller E subfragments. These chimeric DNAs were inserted into vaccinia virus and the expression of E by recombinant vaccinia virus was analyzed. The expressed fusion protein containing 85% of E reacted with hyperimmune dengue virus antiserum by immunofluorescence assay, but was only found intracellularly. In contrast, cell surface expression of chimeric proteins containing a 34, 49 or 60% subfragment of E was demonstrated using antipeptide sera but these proteins did not react with hyperimmune dengue antiserum. Infection of cotton rabbits and rabbits with vaccinia recombinants expressing various chimeric proteins did not induce a detectable E antibody response. Mice immunized with the various vaccinia-chimeric E recombinants were partially resistant to lethal challenge with dengue type 4 virus, but protective efficacy varied markedly in two successive experiments, and a correlation was not observed between surface expression and protective efficacy. Surviving mice exhibited signs of encephalitis. In contrast, a vaccinia recombinant expressing: (i) the 3 structural proteins (C,M,E) plus the first 2 non-structural proteins (NS1, NS2a), or (ii) only the 3 structural proteins, or (iii) only E induced complete resistance to lethal dengue virus challenge and most surviving mice did not develop signs of encephalitis. The latter observations indicate that expression of the dengue virus E and NS1 protective antigens in their mature form on the cell surface is not required for induction of resistance.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00500-02 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Processing and Immunogenicity of Dengue Type 4 Virus Nonstructural Protein NS1		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)		
PI:	Barry Falgout, Ph.D.	IRTA LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section LID, NIAID
	Robert M. Chanock, M.D.	Chief LID, NIAID
	Michael Bray, M.D.	Staff Fellow LID, NIAID
COOPERATING UNITS (if any) Scripps Institute, La Jolla, CA (Lerner); WRAIR, Washington, D.C. (Eckels)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Molecular Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS	1.55	PROFESSIONAL 1.35 OTHER 0.2
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) The nonstructural protein 1 (NS1) of dengue virus has been shown by others to induce protective immunity in mice. The use of NS1 for immunization against infection has one theoretical advantage over immunization with the other dengue protective antigen, the envelope glycoprotein (E). It is possible that antibodies against the envelope protein might cause immune enhancement of disease during subsequent infection by a heterotypic dengue virus. For this reason we initiated a study of the immunogenicity of dengue type 4 NS1. A series of overlapping oligopeptides that span the entire NS1 amino acid sequence were synthesized. In addition, we constructed recombinant vaccinia viruses for expression of: (i) the entire NS1, alone or in combination with other dengue products, or (ii) subfragments of NS1. Analysis of the binding of NS1 specific antibodies to the peptides by ELISA and immunoprecipitation of subfragments of NS1 indicated that the amino terminal third of the NS1 protein contains the major antigenic epitopes. Studies of the expression of NS1 by another series of vaccinia recombinants demonstrated that the hydrophobic sequence preceding NS1 is a functional signal whether it is located at the amino terminus or at an internal position of an expressed polyprotein. In addition, the downstream NS2a sequence was found to be required <u>in cis</u> for the normal cleavage of the NS1/NS2a junction.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00501-02 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Study of the Processing of Dengue Viral Glycoproteins		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Lewis J. Markoff, M.D. Medical Officer LID, NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Molecular Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 0.6	PROFESSIONAL: 0.4	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Processing of the dengue virus pre-matrix (pM) protein was studied during cell-free translation of RNA transcripts prepared from cloned dengue DNA containing capsid, pM, and NH2-terminal envelope glycoprotein (E) sequences. To elucidate the mechanism for processing, strategic mutations were produced in capsid and pM sequences were produced by site-directed mutagenesis of dengue DNA, and mutant RNA transcripts were then translated. Our recent observations suggest that cleavage at the capsid-pM and pM-E sites is mediated by signal peptidase. A signal-like function appears to mediate the translocation event and is linked to cleavage. It is proposed that this function is served by a hydrophobic segment (amino acid residues 100-113 in the dengue precursor) that occurs at the capsid-pM junction. Recognition of the "signal" does not require prior cleavage of NH2-terminal capsid sequences. Our data also suggest that the "signal" has a polar effect on translocation of E, and possibly nonstructural glycoprotein, NS1, sequences.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00502-02 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Antigenic Analysis of the Dengue Virus Envelope Glycoprotein Using Synthetic Peptides		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	Lewis J. Markoff, M.D.	Medical Officer LID, NIAID
Others:	Laraine Henschel Ching-Juh Lai, Ph.D. Robert M. Chanock, M.D.	Microbiologist Head, MVB Section Chief LID, NIAID LID, NIAID LID, NIAID
COOPERATING UNITS (if any) WRAIR, Washington, D.C. (K. Eckels, P. Summers, M.K. Gentry); Research Inst. of Scripps Clinic, La Jolla, CA (R. Houghton, R. Lerner)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Molecular Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS	0.6	PROFESSIONAL 0.4
		OTHER 0.2
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) We identified linear B-cell epitopes in the envelope glycoprotein of dengue virus, type 4, strain 814669, by ELISA using 38 overlapping 15 amino acid peptides that span the 493 amino acid sequence of the envelope as predicted from the nucleotide sequence of cloned DNA. Murine poly- and monoclonal antibodies bound a total of nine peptides. Five of these peptides were weakly bound and represented the N-terminus of the envelope. The remaining four, two of which were bound by monoclonals, were located about 60% of the distance from the N- to C-terminus of the molecule, but were not contiguous with each other. Human sera, largely from patients in endemic areas experiencing a secondary infection, identified five regions in the envelope sequence likely to contain linear antigenic sites. These regions spanned the envelope sequence from N- to C-terminus and were defined by the more frequent binding of 20 of the 38 peptides that were nested in contiguous groups of three to five peptides each. The conditions for interpreting the human data are described. Current and future plans involve use of rabbit anti-peptide antibodies and the use of peptides to define murine and T cell epitopes.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00503-02 LID INACTIVE 1987/88
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Expression of Dengue Virus Antigens Using A Synthetic Signal Peptide.		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Lewis Markoff, M.D. Medical Officer LID, NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Molecular Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 0	PROFESSIONAL: .0	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) One of the major goals of our laboratory is the development of an effective live dengue viral vaccine. A vaccinia recombinant bearing a fragment of the dengue genome coding for all the structural proteins and the major nonstructural protein (NS1) of the virus failed to induce detectable dengue antibodies in experimental animals. It is possible that the lack of immunogenicity of this recombinant was related to failure of the protective dengue viral antigens (envelope, (E), and NS1) to be expressed at the surface of vector virus infected cells. The relationship between cell surface expression and immunogenicity of vaccinia recombinants may be inferred from work done in several other laboratories, as well as our own. Our difficulty in attaining cell surface expression for the dengue antigens is most likely related to the fact that E and the matrix protein (M) are not expressed at the cell surface during the normal course of dengue viral infection. Therefore, processing of E and M coded for by the vaccinia recombinant would not be expected to result in surface expression of the vector coded proteins. The failure of RSV G-dengue E chimeras, including the large fragment of E, to be expressed at the cell surface does not necessarily indicate that this fragment bears signals or conformational constraints that interdict cell surface expression. Instead, the problem may reside in a conformational interaction between the G and E sequences at the amino acid level that results in sequestration in or before the Golgi or the NH₂-terminus of G may determine an unusual pathway through the Golgi to the cell surface that can't be negotiated by the E sequences. To determine whether the large (Hae III) fragment of E included in the first three RSV G-dengue E constructs is able in and of itself to traverse the Golgi and reach the plasma membrane, an attempt has been initiated to express the Hae III fragment of E downstream from a cleaveable signal sequence in an SV40 expression vector. The SV40/HA signal/Hae III E recombinants will then be scored for secretion of the E fragment coded for by the Hae III genomic segment. <u>INACTIVE 1987-88</u>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00531-01 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Functional Analysis of Dengue Nonstructural Proteins, NS3 and NS5		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)		
PI:	Yi-Ming Zhang, M.D.	Visit. Assoc. LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Molecular Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS	0.1	PROFESSIONAL 0.5 OTHER 0.2
CHECK APPROPRIATE BOX(ES)		
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
The dengue virus NS3 and NS5 nonstructural proteins are candidates for viral enzymes that are involved in viral RNA transcription and/or replication. In order to examine the gene expression and functional activity of these two proteins, we engineered NS3 and NS5 specific DNA and constructed a recombinant baculovirus which expressed the NS3 or NS5 viral gene. Synthesis of NS3 in recombinant virus-infected cells was detected by indirect immunofluorescence assay. Similarly, the synthesis of NS5 was detected by SDS-polyacrylamide gel analysis of a lysate from recombinant virus infected cells. The protein products were prepared for immunization of rabbits and antisera specific to these proteins were obtained. The availability of NS3 specific sera should facilitate detection of this protein in experiments designed to study the requirement of downstream NS4a and NS4b sequences for expression of NS3. These experiments will be carried out to test the hypothesis that NS1 and NS3 may be analogous since each is followed by two hydrophobic proteins that may serve as proteases that effect specific cleavage of the dengue polyprotein.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00333-07 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) A Longitudinal Study of Viral Gastroenteritis in Infants and Young Children		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Albert Z. Kapikian, M.D.	Head, Epid. Sec. LID, NIAID
Others:	Kim Y. Green, Ph.D.	Staff Fellow LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
<.1	<.1	<.1
CHECK APPROPRIATE BOX(ES)		
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither		
☒ (a1) Minors		
☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
Rotaviruses have been studied extensively in many parts of the world predominantly by cross-sectional approaches. Such studies have yielded essentially "numerator" data which indicated that rotaviruses are a major cause of diarrheal illness in infants and young children. There has been a paucity of longitudinal viral gastroenteritis studies that yield not only important "denominator" data but also valuable insights into the natural history of a pathogen or illness, with special emphasis on epidemiologic, immunologic and laboratory data. We, therefore, initiated an intensive examination of anal swab and serum specimens obtained during a previous LID long-term longitudinal study (1955-1969) at Junior Village, a welfare institution for homeless, but otherwise normal children. Anal swabs and blood specimens were obtained routinely from the youngest age groups, which ranged from 6 months to about 5 years of age during various periods of the study. Surveillance was carried out by a trained medical staff. Children were housed in cottages according to their age. The scope of the surveillance of about 45-100 infants and young children can be appreciated by the fact that in the 1966 period of the study, a full-time medical staff of 2 nurses and 4 practical nurses from our unit were in daily attendance, in addition to the full-time medical staff of the District of Columbia Department of Public Welfare that included nurses, practical nurses, and a full-time physician in charge of medical care at Junior Village. Rectal temperatures were obtained on each child daily; routinely, a physician examined any child with a rectal temperature of 100.6°F or greater. Careful medical records were kept by the nursing staff on all children. Thus, with this background we plan to investigate the natural history of rotavirus infections in a longitudinal manner.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00335-06 LID
TERMINATED

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (30 characters or less. Title must fit on one line between the borders.)

Rotavirus Reassortants: Genetics and Use in Rotavirus Vaccines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Karen Midthun, M.D. Senior Staff Fellow LID, NIAID

Others: Yasutaka Hoshino, D.V.M. Visiting Scientist LID, NIAID

Jorge Flores, M.D. Visiting Scientist LID, NIAID

Albert Z. Kapikian, M.D. Head, Epidemiol. Sect. LID, NIAID

COOPERATING UNITS (if any)

Flow Laboratories, Inc. (L. Potash)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

0

PROFESSIONAL

0

OTHER

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00338-07 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Cloning of Rotavirus Genes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Jorge Flores, M.D.	Visit. Scientist	LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: .4	PROFESSIONAL: .1	OTHER: .3
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
The analysis of cloned rotavirus genes will greatly enhance our knowledge of the molecular biology of rotavirus. In addition to identification of important regulatory signals (promoters, ribosomal binding sites, etc.), analysis of cloned cDNA can yield information concerning amino acid sequences of structural proteins. Expression of rotavirus antigens by insertion of cloned DNA from VP7 or VP3 into various vectors may provide us with safer RV vaccines. Although rotavirus cDNA clones representing VP7 have been inserted into bacterial and vaccinia expression vectors, the proteins expressed have not been highly immunogenic. It is possible, however, that insertion of the VP3 protein gene into such vectors will result in the expression of a more antigenic protein. VP3 and VP7 clones are being engineered for insertion into adenovirus and bacterial expression vectors which are currently under development. We are currently attempting to transfect rotavirus ss RNA from a given serotype into cells infected with a rotavirus strain of a different serotype in an attempt to recover (under selective pressure) rotavirus reassortants containing the transfected gene. If these experiments yield positive results it will be possible to attempt the rescue of rotavirus modified by <u>in vitro</u> mutagenesis of cloned DNA. Success in developing this methodology will also allow us to probe the function of certain areas of the rotavirus genome (i.e. serotype specific regions in the VP7 gene, cleavage region in the fourth gene, etc.) and could be of considerable help in constructing cross-reactive or further attenuated rotavirus vaccine candidates.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00339-07 LID								
PERIOD COVERED October 1, 1987 to September 30, 1988										
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Isolation and Serotypic Characterization of Human and Animal Rotaviruses										
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 40%;">Yasutaka Hoshino, D.V.M.</td> <td style="width: 20%;">Visit. Scientist</td> <td style="width: 25%;">LID, NIAID</td> </tr> <tr> <td>Others:</td> <td>Albert Z. Kapikian, M.D.</td> <td>Head, Epid. Section</td> <td>LID, NIAID</td> </tr> </table>			PI:	Yasutaka Hoshino, D.V.M.	Visit. Scientist	LID, NIAID	Others:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID
PI:	Yasutaka Hoshino, D.V.M.	Visit. Scientist	LID, NIAID							
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID							
COOPERATING UNITS (if any) Moredun Research Institute, United Kingdom (Snodgrass); Ohio Agricultural Research and Development Center, Ohio (Theil); Texas A & M Univ. (Woode)										
LAB/BRANCH Laboratory of Infectious Diseases										
SECTION Epidemiology Section										
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892										
TOTAL MAN-YEARS	1.2	PROFESSIONAL								
		.4								
		OTHER								
		.75								
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews										
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Bovine rotavirus strain 678, which was originally isolated from a diarrheic calf in Great Britain, was found to belong to a newly established serotype 8 (prototype strain HRV 69M) by plaque reduction neutralization (PRN) assay. Bovine rotavirus strain 1D, which was a "supershort" electropherotype, was found to belong to serotype 6 (prototype bovine rotavirus NCDV strain which has a "long" electropherotype) by PRN assay. Bovine rotavirus strain B223 was shown, by a one-way cross-neutralization assay, to be distinct from any of the 9 established serotypes.										

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00340-07 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Experimental Studies in Animals with Various Rotaviruses and Their Reassortants.		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Yasutaka Hoshino, D.V.M.	Visit. Scientist LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Section LID, NIAID
COOPERATING UNITS (if any) Ohio Agricultural Research and Development Center, Ohio State Univ., Wooster, Ohio (Saif).		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.4	.1	.3
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Continued efforts have been made to delineate the genetic mechanisms underlying pathogenicity of rotavirus in the newborn gnotobiotic piglet model. In these studies the VP3 and VP7 genes have been shown to play an important independent role in rotavirus growth in the gut and in induction of diarrhea. In addition, to these two outer capsid-encoding genes an additional gene (or genes) has been shown to be required for the induction of diarrhea. These findings may have implications for the development of effective reassortant rotavirus vaccines.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00341-07 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Evaluation of Live Attenuated Rotavirus Vaccines		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)		
PI:	Albert Z. Kapikian, M.D.	Head, Epid. Sec. LID, NIAID
Others:	Jorge Flores, M.D.	Visit. Sci. LID, NIAID
	Robert M. Chanock, M.D.	Chief LID, NIAID
	Kim Y. Green, Ph.D.	Staff Fellow LID, NIAID
	Yasutaka Hoshino, D.V.M.	Visiting Scientist LID, NIAID
COOPERATING UNITS (if any) Johns Hopkins U., Balt., MD (M.L. Clements, R. Black, K. Midthun); U. Rochester, NY (R. Dolin); U. Md. (M. Levine); U. of Umea, Sweden (L. Gothevors); U. of Tampere, Finland (T. Vesikari); Vanderbilt U., Nashville, TN (P. Wright); Marshall U., Huntington, W. Va. (R. Belshe); Dept. Med. Res. Min. Health, Rangoon, Burma (K. Moe)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS	2.9	PROFESSIONAL: .8 OTHER: 2.1
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☒ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
Rotaviruses are the single most important etiologic agents of severe diarrhea in infants and young children in developed and developing countries. In developed countries rotaviruses are responsible for one-third to one-half of the hospitalization of infants and young children for diarrheal illness. In developing countries, where diarrheal diseases are responsible for 5-10 million deaths per year in infants and young children, rotaviruses are characteristically the major cause of severe diarrhea in this age group. Thus, the need for a rotavirus vaccine is clear. The goal of such a vaccine is to prevent severe rotavirus diarrhea during the first 2 years of life when the disease is most serious. We have characterized and evaluated an animal rotavirus, the rhesus rotavirus (RRV) strain MMU18006, as a vaccine candidate. RRV has not been recovered under natural conditions from infants or children and thus does not appear to be a virus of the human heritage. In addition, the genes of RRV exhibit significant divergence in sequence from the corresponding genes of the known human rotavirus serotypes. Nonetheless, this virus is similar to human rotavirus type 3 in its neutralization specificity. Furthermore RRV grows efficiently in a semi-continuous line of diploid cells, DBS-FRHL-2. This is an important property because adventitious agents often contaminate primary monkey kidney cell cultures. In addition, RRV can serve as a parent for construction of rotavirus reassortants possessing the major neutralization protein (VP7) of one of the other 3 human rotavirus serotypes. Such reassortants will be needed in a multivalent vaccine since the RRV failed to provide broad protection against other human rotavirus serotypes in the very young infant group.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00343-07 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (50 characters or less. Title must fit on one line between the borders.) The Role of Norwalk Virus and Related Norwalk-Like Viruses in Viral Gastroenteritis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	Kim Green, Ph.D.	Staff Fellow LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Sec. LID, NIAID
COOPERATING UNITS (if any)		
LAB BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS	0.5	PROFESSIONAL .25 OTHER .25
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) Norwalk virus is one of a group of small, noncultivable, 27nm viruses which are important etiologic agents of epidemic gastroenteritis in older children and adults. The adaptation of biotin-avidin immunoassays for detection of Norwalk virus antigen and for detection of seroresponses to the virus allowed us to perform an extensive survey of gastroenteritis outbreaks in Maryland nursing homes for the elderly. Serologic studies indicated that Norwalk virus was associated with gastroenteritis episodes in approximately one-fifth of the patients; this virus was the single most important agent associated with these outbreaks. Attempts to cultivate Norwalk virus are in progress employing a tissue culture system which has been used successfully for cultivation of a porcine calicivirus. In addition, a porcine calicivirus has recently been obtained which will be used as a model for cloning of the Norwalk virus genome.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00346-07 LID	
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Genetic Characterization of Rotavirus by Sequencing and Hybridization Techniques		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Jorge Flores, M.D.	Visit. Scientist	LID, NIAID
Others: Kim Y. Green, Ph.D.	Staff Fellow	LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 0.5	PROFESSIONAL: .25	OTHER: .25
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) An understanding of the molecular epidemiology and the extent and origin of genetic variation of rotaviruses is of basic importance to the development of an effective strategy for immunoprophylaxis of rotavirus disease. The recognition of naturally occurring mutants or reassortants with altered properties (virulence, antigenicity, host range, etc.), may lead to the identification of rotavirus strains of low virulence for infants and newborns which may be suitable for use in a live attenuated virus vaccine. Recently a simple dot hybridization assay and a rapid sequencing technique were developed for analysis of sequences within the genome of rotavirus isolates which may have relevance to immunity or virulence. In this context, the sequences in the VP7 gene of field isolates responsible for serotype specificity or those in the VP3 gene related to rotavirus virulence could be identified and related to the particular clinical and epidemiological features of the infections from which the strains were recovered.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00446-04 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Testing of Rotavirus Vaccine Candidates in Venezuela		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	Jorge Flores, M.D.	Visit. Scientist LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Sect. LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	1.05	PROFESSIONAL: .3 OTHER: .75
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☒ (a1) Minors ☒ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) It is clear from many studies performed in the U.S. and abroad that there is an urgent need to develop a rotavirus vaccine which can be used in both industrialized and developing countries. In an attempt to achieve this goal we have continued our laboratory and field studies of vaccine candidates which include rhesus rotavirus (RRV) strain MMU18006 and reassortants derived from it. RRV is a simian rotavirus strain derived from a stool of a 3.5 month old rhesus monkey with acute diarrhea. It has been passaged 9 times in primary or secondary monkey kidney cell culture and 7 times in DBS-FRHL-2 cell culture, a semi-continuous simian diploid cell strain. The rhesus rotavirus (RRV) appears to be restricted in humans, and its major neutralization protein (VP7) is very closely related antigenically to the corresponding protein of human rotavirus serotype 3. Reassortant rotaviruses with 10 genes from RRV and a VP7 gene from serotype 1, serotype 2 or serotype 4 human rotavirus were prepared in LID and are now being evaluated in phase 1 clinical trials. In phase I and phase II (efficacy) studies in Venezuelan infants the RRV vaccine was shown to be satisfactorily immunogenic and protective. RRV reassortants have also been tested singly or in combination or in combination with RRV in an attempt to induce heterotypic immunity capable of providing resistance to the four commonly circulating rotavirus serotypes.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00450-04 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Relationship of Outer Capsid Protein VP3 of Human and Animal Rotaviruses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Mario Gorziglia, Ph.D. Kazuo Nishikawa, M.D.	Visit. Assoc. Visit. Assoc.	LID, NIAID LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.6	PROFESSIONAL: 0.7	OTHER: 0.9
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The VP3 genes of the simian SA11, bovine NCDV, porcine OSU, and porcine Gottfried strains of rotavirus were studied by sequence analysis. The overall amino acid homology of the VP3 of two SA11 strains was only 82.7%; however the VP3 protein of one of these SA11 strains exhibited an homology of 98.4% with NCDV, suggesting that this SA11 VP3 was derived by inadvertent gene reassortment in another laboratory during contamination with a bovine rotavirus. The VP3 amino acid homology of OSU and other animal rotaviruses is approximately 80%. Surprisingly, the VP3 protein of Gottfried virus is closely related to the VP3 of human asymptomatic rotavirus strains in various ways including (1) nucleotide sequence and (2) neutralization by hyperimmune antisera or VP3 cross-reactive neutralizing monoclonal antibodies. A comparison of the deduced amino acid sequence of the VP3 of two virulent NCDV strains and an attenuated NCDV vaccine strain (RIT 4237), revealed only five amino acid substitutions which were scattered throughout the protein but did not involve the trypsin cleavage sites. One of the virulent strains and the attenuated strain differed in only one amino acid (residue 76) suggesting that a gene or a series of genes other than the VP3 gene sustained mutations which were responsible for attenuation of the candidate RIT vaccine strain of NCDV.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00451-04 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunological and molecular characterization of rotavirus antigens		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	Yasutaka Hoshino, D.V.M.	Visit. Scientist LID, NIAID
Others:	Mario Gorziglia, Ph.D.	Visit. Assoc. LID, NIAID
	Koki Taniguchi, Ph.D.	Visit. Assoc. LID, NIAID
	Kazuo Nishikawa, M.D.	Visit. Assoc. LID, NIAID
	Kim Green, Ph.D.	Staff Fellow LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	0.6	PROFESSIONAL: .2 OTHER: .4
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) In an attempt to further delineate antigenic structure and function of rotavirus we have generated: (i) a series of single gene substitution reassortant rotaviruses and (ii) a series of rotavirus mutants resistant to neutralizing VP7 monoclonal antibodies. Some of the reassortants are potential human rotavirus vaccine candidates. Serologic analysis of the escape mutants and sequence analysis of the VP7 gene of the mutants have revealed a complex structure of neutralization antigenic sites on the VP7.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00478-03 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Development of Adenovirus Expression Vectors		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Jorge Flores, M.D.	Visit. Scientist	LID, NIAID
Others: Gisela Larralde, M.S.	Guest Researcher	LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.4	PROFESSIONAL: 1.3	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Respiratory transmission of adenovirus types 4 and 7 is associated with the development of respiratory illness. However, enteric administration of these viruses produces a silent infection localized to the intestinal tract which is capable of preventing subsequent respiratory illness caused by these viruses. This observation served as the basis for the development in LID of the live enteric adenovirus type 4 and type 7 vaccines. An extensive experience in the use of these vaccines has been gained over the last 20 years since the vaccine is administered routinely to all new recruits in the military. The safety and efficacy of these adenovirus vaccines make them ideal candidates for use as vectors for insertion and expression of foreign genes. Replication of recombinant adenovirus in the intestine should induce an immune response directed at the proteins of the foreign genes and hence confer resistance against the agents from which the genes were derived. The likelihood of the success of this general approach has already been shown by research scientists at Wyeth who were able to express hepatitis B surface antigen by inserting and expressing the corresponding hepatitis B virus gene in the E1 and later in the E3 region of adenovirus type 5. It should be possible to construct adenovirus recombinants that allow packaging of foreign DNA (perhaps up to 5000 bases) after the deletion of non-essential adenovirus regions. We are testing this approach to develop recombinant rotavirus/adenovirus vaccines. The vaccine strains of adenovirus types 4 and 7 vaccines would seem to be safe vectors for foreign genes. Furthermore, infection in the intestines may result in local IgA responses which are particularly important for viruses, such as the rotaviruses, which produce mucosal infection.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00504-02 LID
PERIOD COVERED <u>October 1, 1987 to September 30, 1988</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Sequence of the Sixth Rotavirus Gene</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Mario Gorziglia, Ph.D.	Visiting Associate LID, NIAID
Others:	Yasutaka Hoshino, D.V.M. Robert M. Chanock, M.D.	Visit. Scientist LID, NIAID Chief, LID LID, NIAID
COOPERATING UNITS (if any) BRB, NIAID (L. Maloy)		
LAB/BRANCH <u>Laboratory of Infectious Diseases</u>		
SECTION <u>Epidemiology Section</u>		
INSTITUTE AND LOCATION <u>NIAID, NIH, Bethesda, MD 20892</u>		
TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
25	25	0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) The complete nucleotide sequence of the sixth gene encoding VP6 of two subgroup I animal rotaviruses (vervet monkey rotavirus SA-11 and bovine rotavirus RF) and one subgroup II human rotavirus (Wa) was determined previously. Comparison of the deduced amino acid sequences indicated a high degree of homology; more than 90% of the amino acids were conserved between subgroup I and subgroup II rotaviruses. In the present study we deduced the amino acid sequence of the VP6 protein of 4 additional rotavirus strains, each with a different subgroup specificity: human rotavirus strain 1076 (subgroup I), porcine rotavirus strain Gottfried (subgroup II), equine rotavirus strain H-2 (neither subgroup I nor II) and equine rotavirus strain FI-14 (both subgroup I and II). This allowed us to compare the VP6 amino acid sequences of rotaviruses with dual or no known subgroup reactivity with those of rotaviruses belonging to subgroup I or II. This approach was taken in an attempt to identify epitopes responsible for subgroup specificity. In addition, the importance of the trimeric conformation of the VP6 protein to subgroup epitopes was investigated. Most of the VP6 genes from the different strains contain a unique AUG. In contrast, the VP6 gene of H-2 strain contains an additional AUG at residue 132-134, which results in the translation of a second VP6 protein. Both VP6 products assume a trimeric conformation in the mature virion.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00505-01 LID TERMINATED
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neutralization Epitopes on Rotavirus Outer Capsid Proteins		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Mario Gorziglia, Ph.D.	Visiting Associate LID, NIAID
Others:	Kazuo Nishikawa, M.D.	Visiting Fellow LID, NIAID
	Koki Taniguchi, Ph.D.	Visiting Associate LID, NIAID
	Kim Green, Ph.D.	Staff Fellow LID, NIAID
	Yasutaka Hoshino, D.V.M.	Visiting Scientist LID, NIAID
COOPERATING UNITS (if any) Gamble Institute of Medical research, Cincinnati, Ohio (Ward)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
TERMINATED		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI00506-01 LID
TERMINATED

PERIOD COVERED

October 1, 1987 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Sequencing Rotavirus Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Kim Y. Green, Ph.D.	Staff Fellow	LID, NIAID
Others:	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
	Johnna Sears, M.S.	Biologist	LID, NIAID
	Albert Z. Kapikian, M.D.	Head, Epid. Sect.	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

TERMINATED: Merged with Z01 AI00507-02 LID

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00507-02 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular characterization of rotavirus serotypes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Kim Y. Green, Ph.D.	Staff Fellow	LID, NIAID
Others: Jorge Flores, M.D. Yasutaka Hoshino, D.V.M.	Visit. Scientist Visit. Scientist	LID, NIAID LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Md 20892		
TOTAL MAN-YEARS: 0.6	PROFESSIONAL: .3	OTHER: .3
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The initial objective of this study was to identify serotypic differences by analysis of the nucleotide sequences of rotavirus strains representing different serotypes. Serotype-specific sequences were identified in the gene encoding the VP7 protein and a method for predicting rotavirus serotype from the nucleotide sequence was developed. The identification of serotype-specific sequences has proved to be useful in current studies which have the objectives of (a) developing "serotyping" assays using cloned DNA probes and/or oligonucleotides, (b) analyzing the immune response of individuals to serotype-specific antigens on the VP7 and (c) determining the importance of naturally-occurring variants of each serotype in the epidemiology of rotavirus.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00508-01 LID
TERMINATED

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Naturally Occurring Rotavirus Reinfection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Karen Midthun, M.D.	Senior Staff Fellow	LID, NIAID
Others:	Koki Taniguchi, Ph.D.	Visiting Associate	LID, NIAID
	Albert Z. Kapikian, M.D.	Head, Epidemiol. Sect.	LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS.

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00509-01 LID TERMINATED
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Crossreactive Neutralizing Epitopes on Rotavirus Outer Capsid Protein VP7		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Kazuo Nishikawa, M.D.	Visiting Fellow	LID, NIAID
Others: Mario Gorziglia, Ph.D. Yasutaka Hoshino, D.V.M. Koki Taniguchi, Ph.D. Albert Z. Kapikian, M.D. Robert M. Chanock, M.D.	Visiting Associate Visiting Scientist Visiting Associate Head, Epid. Sect. Chief	LID, NIAID LID, NIAID LID, NIAID LID, NIAID LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<u>TERMINATED</u>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 A1 00510-02 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Rotavirus VP7 Neutralization Epitopes of Serotype 3 Strains		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	Kazuo Nishikawa, M.D.	Visit. Assoc. LID, NIAID
Others:	Mario Gorziglia, Ph.D.	Visit. Assoc. LID, NIAID
	Yasutaka Hoshino, D.V.M.	Visit. Sci. LID, NIAID
	Koki Taniguchi, Ph.D.	Visit. Assoc. LID, NIAID
	Robert M. Chanock, M.D.	Chief LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
0.3	0.3	0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The third serotype of human rotavirus shares neutralization specificity with rotaviruses derived from monkeys, dogs, cats, horses, rabbits, and mice. In addition, viruses of this serotype include strains of all known subgroup specificities (i.e., I, II, neither I nor II, both I and II). The relationships of the major neutralization protein, VP7, of the various serotype 3 strains were analyzed by testing their reactivity with VP7 monoclonal antibodies (mAbs) and by comparing nucleotide and amino acid sequence. This analysis was performed in an attempt to clarify antigenic relationships and to better understand the evolution of serotype 3 rotavirus strains which have established an extraordinarily broad host range.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00511-01 LID TERMINATED
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Rotavirus Cross-Reactive and Serotype-Specific Neutralization Epitopes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Koki Taniguchi, Ph.D.	Visiting Associate LID, NIAID
Others:	Mario Gorziglia, Ph.D. Kazuo Nishikawa, M.D. Yasutaka Hoshino, Ph.D. Albert Z. Kapikian, M.D. Robert M. Chanock, M.D.	Visiting Associate LID, NIAID Visiting Fellow LID, NIAID Visiting Scientist LID, NIAID Head, ES Section LID, NIAID Chief, LID LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<u>TERMINATED</u>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00532-01 LID

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of VP3 and VP7 Genes of Rotavirus Using A Baculovirus Expression Vector

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Kazuo Nishikawa, M.D.
Mario Gorziglia, Ph.D.

Visit. Assoc.
Visit. Assoc.

LID, NIAID
LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.1

PROFESSIONAL:

1.1

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews

☐ (b) Human tissues

☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The VP3 and VP7 outer capsid proteins of rotaviruses are major neutralization antigens and play an important role in the initiation of infection. In order to better understand the function and antigenicity of each protein, we constructed full length cDNA clones of these genes and inserted them into the polyhedrin gene of baculovirus. We plan to carry out the passive immunization studies in the piglet model using antiserum produced with such proteins expressed by baculovirus-rotavirus VP3 or VP7 recombinant.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00533-01 LID
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Analysis of Rotavirus Proteins with Monoclonal Antibodies		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Kim Green, Ph.D.	Staff Fellow LID, NIAID
Others:	Yasutaka Hoshino, D.V.M. Albert Z. Kapikian, M.D.	Visit. Sci. LID, NIAID Head, Epid. Sec. LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20982		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.3	0.3
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) A library of monoclonal antibodies specific for the two neutralization proteins of rotaviruses, VP7 (the major neutralization protein bearing serotype-specificity) and VP3 (the hemagglutinin) was developed in the LID. Efforts have continued in the expansion of this library to include monoclonal antibodies which are needed for vital immunoassays and studies of the antigenic structure of the virus. Seven monoclonal antibodies specific for the new serotype 9 human rotavirus were isolated and characterized. These monoclonal antibodies exhibit high neutralizing activity against serotype 9 viruses but not against other rotavirus serotypes. One monoclonal antibody, 3C3, exhibits strong to serotype 9 viruses in ELISA and is being characterized more extensively prior to its addition to the existing panel of monoclonal antibodies used for serotyping human rotavirus strains. An epitope blocking assay has been developed similar to that described by Shaw et al. and this assay shows promise as a method for measuring serotype-specific responses in vaccinees.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00534-01 LID

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Studies of Non-Group A Rotaviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Kim Green, Ph.D. Staff Fellow LID, NIAID

Others: Albert Z. Kapikian, M.D. Head, Epid. Sec. LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

0.2

PROFESSIONAL:

.1

OTHER:

.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Preliminary studies of a non-group A rotavirus derived from a diarrheic calf indicate that it is distinct antigenically from group A rotaviruses currently studied in this laboratory. This bovine "group B" rotavirus should provide a good model for the development of molecular techniques for analysis of human non-group A rotaviruses.

Laboratory of Microbial Immunity
1988 Annual Report
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SUMMARY STATEMENT

This will probably be the last of these reports. Although LMI has a certain ghostly administrative presence still, its corporeal existence ended, in effect, in October. Dr. B. J. Fowlkes, one of our most productive scientists, was transferred to LCMI. Dr. Asofsky makes most of his administrative arrangements through LI and will be a senior member of that laboratory shortly. Dr. Baker is at present attached to OD, IRP. With luck, the administrative wheels will have ground sufficiently small in a year's time to make these intended changes final.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI-00134-26-LMI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (30 characters or less. Title must fit on one line between the borders.)

Control of Immunoglobulin Synthesis in Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation.)

PI:	Richard Asofsky	Chief	LMI, NIAID
Others:	Hsiao-Kun Chu	Visiting Associate	LMI, NIAID
	David Ennist	Staff Fellow	LMI, NIAID
	Ada Brooks	Biological Lab Technician	LMI, NIAID
	Diana Amsbaugh	Biologist	LMI, NIAID
	Phillip J. Baker	Research Microbiologist	LMI, NIAID
	Michael Fauntleroy	Biologist	LMI, NIAID

COOPERATING UNITS (if any)

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LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Pathology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

5.0

PROFESSIONAL

3.0

OTHER:

2.0

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

FIP4 C3-3 is a hybridoma which secretes both IgM and IgG₃ anti-SSSIII antibodies simultaneously. Immunofluorescent studies indicate that most individual cells are "double secretors." The line has been cloned repeatedly, and is a proved double-secretor because: (1) individual cells stain both for IgM and IgG₃; (2) the line incorporates 14 C lysine into both IgM and IgG₃ *in vitro*; (3) supernates from individual clones in microwells contains both IgM and IgG₃ antibodies to SSSIII; (4) the line produces IgM and IgG₃ paraproteins when transplanted into mice. It was found in the cloning studies that single IgM or IgG₃ producers were fairly common, about 1 in 20.

Molecular analysis with probes for IgM and IgG₃ has been begun. Southern blot analysis of double producing and of IgM producing lines shows no DNA rearrangement thus far. One IgG₃ single producer shows an IgG₃ rearrangement.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-AI-00143-19-LMI
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Genetic Control of the Antibody Response to Microbial Antigens		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI: P. J. Baker Others: J. R. Hiernaux P. W. Stashak M. B. Fauntleroy K. L. Holmes B. Prescott* E. A. Goidl**, S. J. M. McEvoy**	Head, MI, Section Visiting Scientist Microbiologist Biologist	LMI, NIAID LMI, NIAID LMI, NIAID LMI, NIAID BRB, NIAID
COOPERATING UNITS (if any) *Biomedical Research Institute, Rockville, MD 20852; **Department of Microbiology and Immunology, University of Maryland, School of Medicine, Baltimore, MD 21201.		
LAB/BRANCH Laboratory of Microbial Immunity		
SECTION Microbiology and Immunology Section		
INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892		
TOTAL MAN-YEARS: 2.2	PROFESSIONAL: 1.6	OTHER: 0.6
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>RIIIS/J mice</u> make low <u>antibody responses</u> to several <u>polysaccharide antigens</u> of bacterial origin. They have low levels of serum <u>IgM</u> and <u>IgG₃</u>, and high levels of serum <u>IgG_{2a}</u> and <u>IgG_{2b}</u>. Low serum IgM and IgG₃ levels appear to be due to a low frequency of <u>Ly-1 B cells</u>. RIIIS/J mice treated with <u>bacterial lipopolysaccharide (LPS)</u> produce a low anti-bromelain-mouse-erythrocyte (<u>anti-BrMRBC</u>) antibody response, but a normal antibody response against mouse <u>transferrin</u>. These data are consistent with the fact that Ly-1 B cells contain the precursors of <u>B lymphocytes</u> secreting anti-BrMRBC antibody. RIIIS/J mice should be valuable in studies on the development of the <u>Ly-1 B cell lineage</u>.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-AI-00144-24-LMI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of the Antibody Response to Microbial Polysaccharide Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator. Name, title, laboratory and institute affiliation)

PI:	P. J. Baker	Head, MI Section	LMI, NIAID
Others:	J. R. Hiernaux	Visiting Scientist	LMI, NIAID
	P. W. Stashak	Microbiologist	LMI, NIAID
	M. B. Fauntleroy	Biologist	LMI, NIAID
	B. Prescott*		
	J. A. Rudbach**		
	J. L. Cantrell		

COOPERATING UNITS (if any)

*Biomedical Research Institute, Rockville, MD 20852; **Ribi ImmunoChem Research, Inc., Hamilton, MT 59840.

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Microbiology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

2.0

PROFESSIONAL

1.0

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Treatment with non-toxic monophosphoryl lipid A (MPL), derived from a polysaccharide deficient heptoseless Re-mutant of Salmonella typhimurium was found to inactivate suppressor T cell activity as evidenced by a decrease in the degree of low-dose immunological paralysis expressed and an increase in the magnitude of the antibody response to Type III pneumococcal polysaccharide (SSS-III). The effect produced, which could not be attributed to the polyclonal activation of immune B cells by MPL, were dependent upon the dose of MPL used, as well as the time when MPL was given relative to low-dose priming or immunization with SSS-III. Neither amplifier nor helper T cell activity was decreased by treatment with the same--or larger--doses of MPL.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-AI-00145-21-LMI

PERIOD COVERED

October 1, 1987 to September 30, 1988.

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mode of Action of Thymus-derived (T) Suppressor and Amplifier Cells.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	P. J. Baker,	Head, MI Section	LMI, NIAID
Others:	J. R. Hiernaux	Visiting Scientist	LMI, NIAID
	P. W. Stashak	Microbiologist	LMI, NIAID
	M. B. Fauntleroy	Biologist	LMI, NIAID
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COOPERATING UNITS (if any)

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LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Microbiology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.8

PROFESSIONAL:

0.4

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|---|
| ☐ (a) Human subjects | ☐ (b) Human tissues | ☒ (c) Neither |
| ☐ (a1) Minors | | |
| ☐ (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Vicia villosa lectin-adherent Lyt-1^+ spleen cells, obtained 4 days after immunization with an optimally immunogenic dose (0.5 μg) of Type III pneumococcal polysaccharide (SSS-III), increased the magnitude of the antibody response of mice to SSS-III upon transfer to recipients also immunized with the same antigen; however, the ability to demonstrate such enhancement depended greatly upon when such cells were transferred relative to immunization of recipients. Lectin-adherent cells augmented the antibody response of athymic nude (nu/nu) mice to SSS-III, and abrogated the expression--but not the induction--of low-dose immunological paralysis, a form of unresponsiveness mediated by suppressor T cells. These findings are consistent with effects usually attributed to the action of amplifier, rather than contrasuppressor, T cells.

LABORATORY OF MOLECULAR MICROBIOLOGY
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ANNUAL REPORT
LABORATORY OF MOLECULAR MICROBIOLOGY
October 1, 1987 to September 30, 1988

Dr. Malcolm A. Martin, Chief

The Laboratory of Molecular Microbiology (LMM) applies molecular and biological techniques to study the structure, function, and regulation of prokaryotic and eukaryotic genes. Programmatically, a wide range (murine retroviruses, the human immunodeficiency virus (HIV), mycoplasmas, streptococci, and anaerobic bacteria) of microorganisms and their host cells are investigated. Members of the LMM are physically located in two different buildings in Bethesda and in Building 550 at the Frederick Cancer Research Facility. Although the research activities conducted by the LMM staff are quite diverse, a common theme involves the characterization of genetic elements (both chromosomal and episomal) affecting the interaction of microorganisms and the cells they infect.

For the past four years, the LMM staff has made investigations of AIDS and HIV and major research priority. Much of this work has been carried out in collaboration with Drs. Tom Folks, H. Clifford Lane, and Anthony Fauci of the Laboratory of Immunoregulation, NIAID who provide expertise in cell biology and clinical immunology.

The past 5 years has witnessed a tremendous explosion of new information about the biology and molecular structure of HIV. Virtually all of this information has accrued from tissue culture infections of human peripheral blood lymphocytes or continuous T cell lines. We now know that in addition to the gag, pol, and env genes present in other groups of replication competent retroviruses, HIV, like other members of the lentivirus subfamily, contains a number of other genes, many of which regulate the production of viral proteins during the replicative cycle. These include the tat, art/trs, and B/3'orf genes. Studies which have focussed on the biology of HIV infection have demonstrated that T lymphocytes, expressing the CD4 molecule on their surface, can bind virus particles as the initial step in the replicative cycle. Other work has shown that infected tissue culture cells, synthesizing the viral envelope protein gp120, are able to induce the formation of syncytia and death of uninfected T4 lymphocytes.

Although an immense amount of knowledge has been accumulated in a relatively short period of time, precious little information is available about the in vivo life cycle of HIV. At present, little is known about the portal(s) of entry, the type(s) of inocula encountered (free virus versus infected cells), how the virus spreads throughout the body of an infected person (and, for example, how and when it enters the central nervous system), why the virus remains dormant for long periods of time, and the mechanisms of HIV induced disease. A missing component affecting research of HIV is the absence of a tractable animal model. Only two animal species are naturally susceptible to infection with HIV (man and the chimpanzee) and only man develops disease. Although several other lentivirus systems appear promising (such as the simian immunodeficiency virus [SIV]) as of a model for retrovirus induced immunodeficiency, in the final analysis, extrapolations back to HIV will invariably have to be made.

For the past two years we have contemplated the use of transgenic mice to study different phases of HIV infection in vivo. The construction of transgenic animals that would permit an evaluation of the entire life cycle of HIV to occur would be ideal. Such a system would allow the dissection of the multiple steps required for efficient in vivo infection and disease production. Our first thoughts turned to the construction of mice

expressing the human CD4 protein, the receptor for HIV. Unfortunately, work from other laboratories demonstrated that although mouse L cells, NIH 3T3 cells, and several mouse lymphocyte lines could be induced to synthesize human CD4 (following stable transfection), no evidence of HIV infection could be demonstrated when these cells were subsequently exposed to virus. Virus bound to the T4 positive mouse cells but failed to enter and initiate a replicative cycle. Exchanges of the mouse and human CD4 cytoplasmic "tails" did not alleviate the block in susceptibility. The production of mice expressing human T4 therefore seemed to be impractical as a model system for HIV.

HIV infection in man is characterized by months to years of inapparent disease before development of AIDS or AIDS-related syndromes. During this interval, free virus is extremely difficult to demonstrate. It is very likely that this inefficient expression of HIV reflects, in part, the presence of latent forms of virus in mononuclear cells. Populations of infected lymphocytes or macrophages may exist which harbor integrated, but inactive HIV proviruses. Such potential cell reservoirs of virus could initiate rounds of virion production if appropriately stimulated. Factors capable of activating dormant copies of proviral DNA might also contribute to disease progression. An animal model that simulates latent HIV infection of man could facilitate the study of relevant cofactors of disease progression. Toward this end, two types of transgenic mice carrying different portions of HIV proviral DNA were constructed.

Four strains of transgenic mice were created containing the HIV long terminal repeat (LTR) linked to the bacterial gene, chloramphenicol acetyl transferase (CAT) in a collaboration with Dr. Heinrich Westphal, NICHD. A survey of tissues from progeny of each strain indicated similar patterns of CAT expression. The highest levels of CAT were detected in thymus, eye (lens epithelium), heart and tail. Extracts of spleen and blood showed low levels of CAT expression. No constitutive CAT was detected in lung, brain, skeletal muscle, kidney, tongue, colon, thyroid or bone marrow. One of the tissues that was consistently positive for CAT was the tail. Expression in tail specimens was localized to the skin and further fractionation revealed CAT activity in the epidermal layer. When epidermal cell suspensions were separated into keratinocyte and Langerhans cell fractions, very high levels of CAT were detected only in the latter.

Langerhans cells are dendritic antigen-presenting mononuclear phagocytes by several antigenic, morphologic and functional criteria. They originate in the bone marrow and continually repopulate the epidermis. Our data indicate that CAT expression in Langerhans cells was more than a 1000-fold higher than that observed in circulating monocytes and more than 10-fold greater than seen in bone marrow derived macrophages stimulated with several recombinant cytokines. HIV LTR-directed CAT synthesis in transgenic mice appears to parallel differentiation of cells of monocyte-macrophage lineage and most likely is responsive to the expression of regulatory proteins specific to these cells.

During the course of these studies, experiments were carried out in which an infectious molecular cloned HIV provirus was transfected into several human and non-human cell lines. In virtually all cases, including those in which the cloned viral DNA was introduced into mouse 3T3 cells, infectious progeny HIV particles were generated that exhibited the full spectrum of biological properties. This result suggested that no intrinsic barrier to the synthesis of HIV proteins and their assembly into infectious virions existed in mouse cells. In view of our findings with transgenic animals containing the HIV LTR element, we surmised that mice harboring an intact, biologically

active copy of the HIV genome might synthesize viral proteins and virions. If either of the latter were toxic to the mouse, a possible "disease" model was in the offing.

In the fall of 1987, members of the LMM staff petitioned the NIH Biosafety Committee for permission to construct transgenic mice carrying complete copies of HIV proviral DNA. In November, 1987, the experiment was approved with the stipulation that it be conducted in the BL4 facility of Building 41T. Microinjections, carried out by Dr. Jan Abramczuk, NIDR commenced shortly thereafter and the first animals were born in December 1987. To date, 13 founder transgenic animals have been born, 5 of which transmit copies of the HIV provirus to their progeny. The founder animals contain 2 to >64 copies of HIV proviral DNA/cell, have shown no evidence of disease, and produce no detectable progeny virions.

One of the 5 founder animals (female mouse 13) containing 2 copies of proviral DNA, was particularly interesting. Besides developing antibodies directed against gp120 and the p64 reverse transcriptase protein of HIV, mouse 13 produced litters which developed a fatal disease syndrome, similar in many respects to human AIDS. Since January 1988, approximately one-half of the animals in 5 different litters from mouse 13 have exhibited the unique disease phenotype; non-transgenic littermates were perfectly healthy. Affected animals were apparent by day 10 to 12 of life by their small, runted appearance coupled with characteristic dry, scaly, and fissured tails, ears, and paws. In general all of the affected animals were dead by day 28 of life.

Several of the affected animals were autopsied to evaluate the location and extent of diseased tissues. All animals had extensive lymphadenopathy affecting brachial, inguinal, cervical, pancreatic and maxillary nodes. Microscopically, the lymph nodes showed evidence of hyperplasia with increased numbers of B lymphocytes (see below). Striking changes were also observed in the lungs of the affected animals. Animals sacrificed early in the course of the disease, had areas of lymphoid aggregation localized almost exclusively to regions surrounding the pulmonary blood vessels. At later times, the lymphoid infiltrates extended out from the regions of the vasculature into the pulmonary interstitium. In the most advanced situations, the lymphoid aggregates obliterated the alveolar spaces from without. In no case was there evidence of bronchopneumonia which might suggest a bacterial infection. Histologic examination of the skin from affected areas revealed marked hyperkeratosis and pronounced parakeratosis associated with infiltrating granulocytes at the dermal-epidermal junction. This constellation of findings is reminiscent of human psoriasis, a condition frequently encountered in AIDS patients.

Attempts to isolate virus were carried out by co-cultivating sterile tissue specimens with T4 positive human lymphocytes. Virus was isolated from the skin, lymph nodes, and spleen of three different affected mice. Immunoblot analysis of the recovered virus that had been passaged a single time in human T cells, indicated a protein banding pattern that was indistinguishable from the HIV parent. The results of preliminary experiments indicate no alteration of the host range (viz. exclusively human CD4 dependent) of the rescued virus.

Lymphocytes recovered from spleen and lymph nodes of affected mice were sorted by FACS. No depletion of the Leu3/T4 subset of T cells was observed in affected animals compared to their healthy, non-diseased littermates. However, the affected animals consistently had a marked increase in B cells which will be investigated further. T cell functional assays are also in progress.

The spontaneous disease appearing in progeny of mouse 13 shares many features with AIDS in humans. The lymphadenopathy, lung disease characterized by lymphocytic accumulations around pulmonary vasculature, psoriaform skin lesions, virus isolation, and death have been frequently reported for HIV seropositive individuals. Of particular note is the development of disease in the transgenic animals without any obvious reduction of T4 cells. This suggests that other cell types, perhaps those of monocyte/macrophage lineage, are responsible for the pathologic changes observed. We are planning to develop this model more extensively over the next year and use it for investigating: 1) mechanisms underlying HIV induced cell and tissue injury; and 2) potentially useful agents that inhibit or delay disease development.

Other Research Accomplishments

Identification of a novel HIV gene that is expressed in vivo. A 16 kilodalton protein, expressed in HIV-1 producing cells, was identified as the gene product of the viral "U" open reading frame (ORF). When expressed *in vitro*, the 81 amino acid *vpu* protein reacted with approximately one-third of the serum tested from HIV seropositive individuals. A mutation in the U ORF resulted in a 5- to 10-fold reduction of progeny virus particles but little if any effect on the quantity of intracellular viral proteins. These results indicate that the *vpu* protein is required for efficient virus replication and may have a role in assembly or maturation of newly synthesized particles (Strebel, Klimkait, and Martin).

In vitro mutagenesis identifies a region of the HIV gp120 that is critical for infectivity. Site-specific mutagenesis was used to introduce amino acid substitutions within the second highly conserved domain of the HIV gp120. One of the changes amino acid 267 abolished infectivity but had no effect on the binding of mutant particles to cells expressing the CD4 receptor or the production of syncytia by mutant virus producing cells. These results suggest that alterations of the second conserved domain of gp120 interferes with a critical early step in virus replication other than receptor binding and fusion (Willey and Martin).

Identification of functionally important regions of HIV gp41. *In vitro* mutagenesis was used to introduce hydrophilic amino acids into the highly conserved and strongly hydrophobic N-terminus of gp41. When the Phe-Leu-Gly motif was disrupted by such procedure, the virus was no longer infectious nor capable of eliciting syncytia. A termination codon, similar in location to that present in the gp37 of HIV-2, was introduced into sequences encoding the cytoplasmic tail of HIV-1 gp41. Proviruses carrying this alteration were no longer infectious (Klimkait, Willey, and Martin).

Emergence of replication-competent revertants of HIV env gene mutants. Some HIV mutants, rendered non-infectious following the introduction of alterations in either gp120 and gp41, regained their ability to replicate in human T cells following long term passage in culture. Molecular cloning of the revertant proviral DNAs and nucleotide sequencing analyses revealed that the original mutations remained and compensatory alterations in neighboring regions of the HIV envelope protein restored biological activity (Willey, Klimkait, and Martin).

Biosynthesis, cleavage, and degradation of the HIV envelope glycoprotein gp160. The synthesis and processing of HIV gp160 and gp120 was studied in pulse-chase experiments during virus replication in a T4 positive lymphocyte cell line. Only 10 to 20% of the originally synthesized gp160 could be chased to gp120. Efficient intracellular

sorting results in the transfer of uncleaved gp160 to lysosomes where it is degraded while gp120 is transported to the cell surface and secreted. Cleavage of the precursor gp160 to generate gp120 occurs intracellularly and can be inhibited by ammonium chloride (Willey, Klausner, Bonifacio, and Martin).

Infection of human monocyte cultures in vitro. Procedures were established for preparing and propagating peripheral blood monocyte cultures that are susceptible to HIV infection. Characterization of a virus stock that replicated efficiently in monocyte cultures indicated that it consisted of a least two components: 1) a macrophage-tropic virus and 2) virions that can replicate in both lymphocytes and macrophages. Pure stocks of each are being prepared and examined both biologically and molecularly for the identification of determinants of cell tropism (Potts and Martin).

Genetic recombination of the human immunodeficiency virus. A clonal cell line (8E5), carrying a single reverse transcriptase-defective integrated provirus, was used in transfection studies with different defective mutants derived from an infectious molecular clone of HIV. Infectious viral particles were recovered following transfection of 8E5 with a tat mutant and an env mutant. Several candidate recombinant viruses obtained from these transfection experiment were plaque-purified. Restriction analysis of proviral DNA from these viruses indicated that they emerged through genetic recombination (Clavel, Hoggan, Martin, and Repaske).

Functional analysis of HIV B (3') ORF protein. The 3'(B)-orf product of HIV-1 was shown to be a negative regulator of virus replication. B orf mutant proviruses replicated more vigorously than wild-type HIV proviral DNA during transient expression in a variety of nonlymphoid and lymphoid cell lines. The HIV B orf product, supplied in trans as an expressible cDNA, suppressed, in a dose-dependent manner, the replication of wild type virus and enhanced the replication of B orf mutant proviruses. Transient expression of reporter genes that were linked to the HIV LTR was also repressed by coexpression of B orf linked to HIV LTR or the CMV immediate early promoter. The negative effect of B orf was mediated by a cis sequence, designated the negative regulatory element which is situated between 340 and 156 nucleotides upstream of the RNA initiation site within the HIV LTR (Ahmad and Venkatesan).

Interactions of HIV B and art/trs gene products. The art/trs protein of HIV was shown to "supertransactivate" the expression of a replication-incompetent, art negative proviral DNA (pNL432-260). This supertransactivation was accompanied by 1) brisk expression of env gp 160 and gag proteins, 2) increased levels of all species of viral RNAs, 3) complete shutdown of the HIV B orf expression. The magnitude of transactivation of mutant provirus was relatively constant over a wide range of arts/trs cDNA concentrations (0.5-20 µg). When increasing amounts of B orf cDNA was transfected into cells, a dose dependent repression of virus production from both wild-type art/trs mutant proviruses was observed. The B orf induced repression could not be alleviated by coexpression of ars/trs cDNA (Ahmad and Venkatesan).

Structural analysis of HIV gag-pol protease. The p64 and p51 reverse transcriptases and the p34, the integrase proteins of HIV have been previously mapped and sequenced. This analysis of HIV pol gene products has been extended to include the identification and sequencing of a p10 gag-pol protease, the enzyme responsible for the processing of the gag and gag-pol fusion proteins. A 10 kDa protein with an identical N-terminal sequence (determined through the first 39 residues) was present among the processed HIV pol gene products in E. coli expressing the entire HIV pol open reading frame. Although

the purified viral protease lacked measurable proteolytic activity, bacterial extracts were capable of processing an HIV gag precursor protein synthesized in *E. coli*. A sensitive assay for the HIV protease using defined oligopeptides has been developed and will be employed to screen protease inhibitors as potential antiviral drugs (Venkatesan).

Syncytia assay for the HIV envelope protein. One manifestation of HIV infection *in vitro* is formation of syncytia that involves virus infected cells and cells expressing the CD4 protein. A HeLa cell line expressing CD4 was transfected with HIV envelope expressing derivatives of the infectious provirus clone, pNL4-3. Syncytia were observed 24 to 48 hours later. A variety of HIV *env* mutations were tested in this assay to ascertain which portions of the HIV envelope mediated the formation of syncytia. Mutations in the CD4 binding region of gp120 and the amino terminus of gp41 eliminated syncytia, and were non-infectious when introduced into the infectious clone pNL4-3 (Felser, Klimkait, Martin, Silver).

Activation of the HIV LTR by herpes simplex virus. Infection of cells by herpes simplex virus type 1 (HSV-1) results in activation of the HIV LTR. Deletion mutagenesis studies suggest that the region of the HIV LTR responsive to HSV activation included a DNA segment containing the binding sites for the cellular transcriptional factors NFκB and Sp1. Exonuclease protection assays, designed to map the sites of nuclear protein the HIV LTR DNA, demonstrated increased binding to a segment of the LTR containing the NFκB and Sp1 sites in HSV-infected HeLa cells. Gel retardation studies showed that HSV infection of HeLa cells results in the induction of NFκB binding activity. The induction of this DNA-binding factor may be important, not only for the activation of HIV by HSV, but also may regulate of HSV gene expression (Rabson, Ostrove, Gendelman, Gimble, and Max).

Cytokine-induced activation of HIV expression in a chronically infected promonocyte cell line. A number of approaches have been utilized to study the mechanisms by which the expression of integrated HIV proviruses in a cloned promonocyte cell is induced by cytokine treatment. The integrated proviruses present in the cell line (U1 cells) was molecularly cloned, and the expression of one was shown to be induced by cytokine treatment. DNA binding studies have shown that the activation of HIV expression by cytokines in U1 cells is associated with the induction of protein which interacts with both the NFκB and Sp1 sites in the HIV LTR. These studies will permit a greater understanding of the molecular mechanisms underlying the activation of which latent HIV proviruses in cells of the monocyte lineage (Rabson, Maury, Clouse, Folks, and Fauci).

Infection of human placental tissue by HIV-1. CD4 protein can be demonstrated by immunocytochemistry on a number of different cells in the term human placenta, including cells lining the perimeter of chorionic villi, most likely of trophoblastic origin, cells within the villous stroma (possibly placental macrophages and endothelial cells. Organ cultures of first trimester and term human placenta can be infected by HIV-1 as demonstrated by production of infectious viral particles and expression of viral antigens within cultured placental cells. These studies demonstrate that human placental tissue is directly infectable by HIV and, thus, demonstrate a potential route of in utero transmission of HIV from an infected mother to her offspring (Maury, Potts, and Rabson).

Transcriptional activity of non-ecotropic MuLV sequences in wild mice. *M. spretus* (Morocco) contains 8 or more copies of *env* sequences related to the 5' *env* segment of the MCF MuLV. No infectious virus is produced by these mice and Northern blot analysis of RNAs extracted from various tissues contain no detectable retrovirus-related

RNAs. However, infectious MCF virus could be isolated 5 weeks after inoculation with ecotropic MuLV indicating that these endogenous sequences could contribute to the generation of replication competent MCF MuLVs. One full-length M. spretus provirus has been molecularly cloned and the LTR partially sequenced. The U5 and R regions of this LTR resemble those of other MuLVs, but major differences were observed upstream and downstream of the direct repeat region. This LTR has no promoter activity despite the presence of a TATAA box, and experiments are underway to determine if any negative regulatory proteins repress its function (Villar, Kozak).

Characterization and sequencing of a novel leukemogenic MuLV from *Mus hortulanus*. A novel infectious MuLV was isolated from the wild mouse species M. hortulanus. HoMuLV is a NB-tropic ecotropic MuLV which causes lymphomas in female NIH Swiss mice with a 5-10 month latency, and erythroleukemia and myelogenous leukemia in male Swiss mice with a latency of 12-16 months. Sequencing of the LTR, gag, and env regions of HoMuLV confirmed that it differs significantly from other known murine retroviruses. Major differences were noted in the gp70 which was only 65% homologous to other ecotropic MuLVs, and in the p12-gag which was less than 60% homologous to that of other viruses. Hybridization studies, employing the 5' region of the HoMuLV gp70 as a probe, clearly show that HoMuLV is an exogenous infectious agent which is not transmitted as a germline provirus in any strain of mice, including Hortulanus mice (Voytek and Kozak).

Genetic mapping of mouse chromosomal genes. Southern blot analysis of DNAs extracted from hamster x mouse somatic cell hybrids have been used to chromosomally map various genes in the mouse with particular emphasis on genes involved in oncogenesis. Newly mapped genes include those encoding the tumor rejection antigen gp96, a tumor-specific integration site in Abelson MuLV induced lymphoma, the Fms and Met proto-oncogenes, a sequence rearranged with Igk in a mouse myeloma, and a sequence which may represent the ecotropic MuLV cell surface receptor. Hybrid cell lines have also been used to map other mouse genes including those for various lymphoma cell surface antigens, spermatid associated proteins, genes related to *Drosophila* heat shock proteins, and the mouse homologue for the Huntington's disease marker. A subtractive cloning procedure was used to isolate Purkinje cell specific cDNAs and these were chromosomally mapped to 3 chromosomes containing neurological markers known to affect Purkinje cell development. Finally, hybrid cells, containing single mouse chromosomes, have been used to prepare a gene library specific for chromosome 16 (Kozak).

A putative negative control region in MuLV LTRs. Two novel protein binding sites have been identified near the 5' terminus of all MuLV LTRs, upstream of the enhancer region. These sites were present in a highly conserved sequence designated as the upstream conserved region (UCR) and bound nuclear factors designated as UCRF-L and UCRF-U. The essential binding sequences of these two murine factors were found to be distinct but overlapping. Northern analysis of five different mouse strains revealed an inverse correlation between MuLV RNA expression and UCRF-L activity, thus suggesting a negative regulatory function of UCRF-L (Khan, Krieg, and Flanagan).

Expression of endogenous retroviral-related sequences in mammalian tissues. Brain, liver, and kidney tissues of mouse and man were analyzed for expression of MuLV-related and endogenous human (4-1-related) retroviral sequences, respectively. In both cases, tissue-preferred expression was seen: kidney > brain > liver. RNAs were expressed throughout life (18 day embryo to 28 months of age in case of the Mus musculus wild

mouse and an 8 months to 85 years of age in man) and increasing with age. These results may indicate a relaxation of endogenous retroviral gene expression in mammalian cells with aging (Khan and Cuttler).

Novel application of polymerase chain reaction used to clone flanks of integrated proviruses. Mouse genomic DNA was cut with *Sau3A* and the small fragments circularized by ligating dilute solutions of DNA. Some of these DNA circles contain the end of a long terminal repeat (LTR) sequence linked to flanking cellular DNA. The *Sau3A* circles were then restricted with *PstI* which cuts near the end of the LTR, and hence linearizes circles containing LTR plus flank, leaving bits of LTR sequence on either side of what was originally flanking cellular DNA. The PCR technique was then used to amplify these fragments. (Silver, Keerikatte)

Monoclonal antibodies against ras proteins. Monoclonal antibodies (Mabs) against intact *ras* proteins were prepared and the region of the p21 molecule encompassing the epitope recognized by each antibody was determined. Two regions (amino acids 23-69 and 89-106) accounted for the epitopes of 80% of the Mabs raised, indicating that those are the regions most exposed at the molecular surface and thus the most immunogenic. These Mabs exhibited significantly higher affinity than other known *ras* antibodies and permitted their use for immunoaffinity purification of *ras* proteins and immunohistochemical studies of fixed tissues (Nebreda, Alonso, Bryan, Santos).

Quaternary structure of ras proteins. The use of radiation inactivation techniques, molecular cross-linking, and sedimentation analyses indicated that *ras* gene products are organized as homo-oligomers. This finding has important implications for the role of these proteins in signal transduction indicating that *ras* proteins do not conform to the structure of monomeric alpha subunits in classical G proteins and suggest that activation of p21 occurs through allosteric subunit reorientation triggered by guanine nucleotide binding (Nebreda, Bryan, Santos).

Ras gene products and the inositol-lipid signalling pathway. Specific alterations were observed in the PI cycle of *ras*-transformed fibroblasts, that were similar to changes effected by spontaneous transformation or transformation mediated by either membrane-associated oncogenes (*src*, *met* or *trk*) or the cytoplasmic oncogenes *mos* and *raf*. The nuclear oncogenes, *fos* and *myc*, did not produce these changes. The alterations observed included: (a) stimulation of phospholipase A2 activity and (b) specific uncoupling of surface receptor-mediated stimulation and activation of intracellular phospholipase C. These findings suggest the existence of common biochemical pathways for transformation by cytoplasmic and membrane-associated oncogenes and are in conflict with the notion that p21 *ras* proteins are G-like regulatory elements of phospholipase C or phospholipase A2 in PI signal transduction pathways. (Alonso, Santos)

Ras proteins and cellular phosphorylation. Maturation of *Xenopus* oocytes induced by microinjected *ras* proteins is associated with phosphorylation of specific cellular substrates. *Ras* proteins induced the phosphorylation of specific proteins that were not present in oocytes treated with either progesterone or insulin. Furthermore, unlike maturation and phosphorylation triggered by those hormones, microinjected *ras* proteins produced their effects in the absence of protein synthesis. These observations indicate that *ras* proteins participate in signal transduction by triggering phosphorylation of specific cellular substrates and suggest that they may regulate cascades of phosphorylation involved in control of the cell cycle (Nebreda, Santos).

ADMINISTRATIVE CHANGES

During the past year three members of the LMM scientific staff left NIH to assume faculty appointments or positions at other research institutions. Dr. Donald Le Blanc was appointed Professor of Microbiology, University of Texas, San Antonio. Dr. C. Jeffrey Smith assumed a faculty appointment in the Department of Microbiology and Immunology, Medical School, East Carolina University in October 1987. Dr. Howard Gendelman, an Expert in LMM for two and a half years, was appointed to the staff of Walter Reed Army Institute of Research in November, 1987, where he will continue his investigations of HIV infection of monocytes.

Dr. John Silver was advanced to tenure status in the LMM.

Three post-doctoral fellows joined the Laboratory for research training including Drs. James Felser, Eliza Vicenzi, and Benjamin Berkhout. Dr. Teh Jaeng, a Senior Staff Fellow from NCI with research interests involving HTLV I and HIV gene regulation, joined LMM in August, 1988.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZOI A1 00027-21-LMM

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Basic Studies of Mycoplasmas

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)

PI: Joseph G. Tully Head, Mycoplasma Section LMM, NIAID

Others: David L. Rose Research Microbiologist LMM, NIAID

COOPERATING UNITS (if any)

J.B. Baseman, Univ. Texas, San Antonio; J.M. Bove, Univ. Bordeaux, France; R.F. Whitcomb, USDA, Beltsville, MD; J. Boslego, Walter Reed Inst. Res., Washington, D.C.; G. H. Cassell, Univ. Alabama, Birmingham.

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Mycoplasma Section

INSTITUTE AND LOCATION

NIAID, NIH, Frederick, Maryland 21701

TOTAL MAN-YEARS

3.0

PROFESSIONAL

1.0

OTHER

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Recent efforts have continued in characterization of the biological and molecular features of Mycoplasma genitalium, in anticipation that this information will lead to development of improved methods in detection of the organism and more definite confirmation of its role in human disease. Mycoplasma genitalium, first isolated from the urethra of two men with nongonococcal urethritis in our laboratory in 1980, has now been found retrospectively in throat specimens of military recruits participating in an inactivated M. pneumoniae vaccine field trial in 1974-75. Three of sixteen preserved throat isolates, previously identified as M. pneumoniae, have now been shown to be mixtures of M. pneumoniae and M. genitalium. Purification of these mixed mycoplasmas by selection of single colony isolates clearly confirmed the presence of M. genitalium. Identification of M. genitalium was based upon the occurrence of a species-specific 140 kDa protein adhesin in these isolates and their extensive serologic reactivity to a M. genitalium antiserum. The frequent occurrence of both M. genitalium and M. pneumoniae in a number of these throat specimens, in combination with the shared serologic reactions between these two mycoplasmas, suggests the likelihood that M. genitalium strains might be easily missed in current diagnostic laboratory techniques. What role M. genitalium might have in human respiratory disease remains to be determined. Further studies on other mollicutes, primarily of insect origin, have led to description of new species of Acholeplasma, the isolation of acholeplasmas from human amniotic fluid, revised reclassification of helical mollicutes (spiroplasmas), and an enlarging group of other mollicutes that have been difficult to assign to established taxonomic groups.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00190-10-LMM
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Molecular Genetics of Eukaryotic Cells and Their Viruses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI: Malcolm A. Martin Others: John Leonard Howard Gendelman Barbara Potts	Chief Medical Staff Fellow Expert Senior Staff Fellow	LMM, NIAID LMM, NIAID LMM, NIAID LMM, NIAID
COOPERATING UNITS (if any) Thomas Folks, Expert, LIR, NIAID; Heinrich Westphal, LMG, NICHD; Jan Abramczuk and Abner Notkins, LOM, NIDR; Monte Meltzer, WRAIR; Gene Shearer and Fran Hakim, Immunology Branch, NCI.		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Biochemical Virology		
INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892		
TOTAL MAN-YEARS: 7	PROFESSIONAL: 3	OTHER: 4
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Two types of transgenic mouse systems were developed for investigations dealing with the <i>in vivo</i> expression of human immunodeficiency virus (HIV) gene products. The first utilized the HIV long terminal repeat linked to the bacterial gene, chloramphenicol acetyl transferase (CAT). The four transgenic strains obtained all exhibited spontaneous expression of CAT in the thymus, lens epithelium, spleen, heart, and the skin. Fractionation of ear skin from transgenic animals indicated that very high constitutive levels of CAT activity occurred in Langerhans cells. The latter are highly differentiated dendritic macrophages which comprise 2 - 5% of epidermal cells and actively produce virus in HIV positive individuals. Transgenic animals were also constructed which contain intact copies of the HIV proviral DNA. Progeny, resulting from the mating of one of the 5 founder animals to a non-transgenic mouse, develop an AIDS-like syndrome consisting of lymphadenopathy, a lung disease characterized by accumulation of lymphocytes around blood vessels, a psoriasis-like skin disease, retarded development, and death by day 28 of life. Non-transgenic littermates never develop the disease syndrome. HIV has been recovered from skin, lymph nodes and spleen. Immunologic studies indicate no depletion of the Leu 3/T4 subset of mouse T cells in the affected animals.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI-00218-07-LMM

PERIOD COVERED

October 1, 1987 to September 30, 1988

*TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical and Chemical Studies on Retroviral DNA.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Roy Repaske	Research Chemist	LMM, NIAID
Others:	Francois Clavell	Visiting Fellow	LMM, NIAID
	Sundarajan Venkatesan	Expert	LMM, NIAID
	M. David Hoggan	Senior Scientist	LMM, NIAID
	Ron L. Willey	Biologist	LMM, NIAID
	Malcolm A. Martin	Chief	LMM, NIAID

COOPERATING UNITS (if any)

Janet Hartley, Research Microbiologist, LVD, NIAID; Richard J. Feldmann, CR, DRT

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

3

PROFESSIONAL

2

OTHER

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Six conserved amino acids are found in an alignment of *E. coli* RNase H with the C-terminal region of Moloney murine leukemia virus (M-MuLV) reverse transcriptase (RT) and other retroviral pol sequences. Functional significance of selected amino acids in the putative RNase H domain of reverse transcriptase (RT) was determined in partially purified cloned M-MuLV RT expressed in *E. coli* and in infectious molecularly cloned M-MuLV after amino acid substitutions were made by site-directed mutagenesis. Modification of either of two conserved aspartic acid residues resulted in greater than 25-fold loss of RNase H activity. One of these mutant enzymes exhibited normal RNA dependent polymerase activity, whereas polymerase activity (dTTP and dGTP incorporation) was reduced 50% in the other. MuLV proviruses containing either of these modifications were replication defective. Infectious ecotropic virus phenotypically similar to M-MuLV could be rescued by co-infection or co-transfection with competent amphotropic or MCF MuLVs. These findings support the localization of the RNase H domain in RT, show that a single amino acid substitution can selectively suppress RNase H activity, and confirm the requirement for RNase H for viral replication. Genetic recombination between two defective Human Immunodeficiency Virus (HIV) sequences has been demonstrated. Three different replication defective proviruses constructed by site-directed mutagenesis of the *tat*, *int*, or *env* regions of an infectious HIV molecular clone were used. A cell line, 8E5, carrying a pol defective endogenous HIV provirus was transfected with each of three mutants. Infectious virus was recovered after transfection with *tat* or *env* mutants and were plaque purified. Restriction mapping of viral DNA isolated from cells infected with these biologically cloned recombinant viruses indicated multiple crossing over events had occurred to generate infectious virus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI-00219-06-LMM
PERIOD COVERED October 1, 1987 to September 31, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular and Genetic Analysis of Streptococci		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	D. J. LeBlanc	Head, BVS
Others:	L. N. Lee E. Zito	Chemist NRC Postdoctoral Fellow
		LMM, NIAID LMM, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Bacterial Virulence Section		
INSTITUTE AND LOCATION NIAID, NIH, Frederick, Maryland 21701-1013		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES)		
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither		
☐ (a1) Minors		
☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)		
Project Terminated		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 A1-00222-06-LMM

PERIOD COVERED

October 1, 1987 to September 31, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Endogenous Ecotropic and Xenotropic Murine Leukemia Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	T. Theodore	Research Microbiologist	LMM, NIAID
	A. S. Khan	Senior Staff Fellow	LMM, NIAID
	M. A. Martin	Chief	LMM, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|--------------------------------------|
| ☐ (a) Human subjects | ☐ (b) Human tissues | ☐ (c) Neither |
| ☐ (a1) Minors | | |
| ☐ (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project Terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00281-07 LMM
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Molecular Cloning of Recombinant MuLV Proviral Sequences in Inbred Mice		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: Charles E. Buckler Research Biologist LMM, NIAID Others: Christine A. Kozak Research Microbiologist LMM, NIAID		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory Of Molecular Microbiology		
SECTION Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Utilizing a variety of analytical methods (statistical, numerical, and sequence homology studies) an evaluation of the variation in retroviral proteins produced by both murine and human (HIV) viruses has been performed. These studies have attempted to define conserved and variable regions of both the murine and human viral genes. In this way portions of retroviral genes that might be essential for viral replication (i.e. conserved) have been identified. These regions are candidate areas for mutagenesis studies directed toward the understanding of and the intervention in viral replication. Microcomputer software has been developed to allow a rapid and accurate analysis of gene mapping studies. When applied to data obtained in studies utilizing DNA from mouse-CHO hybrid cell lines successful results were obtained.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-AI-00300-07-LMM

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Control of Resistance to Viral Leukemogenesis in Wild Mouse Populations

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: C. A. Kozak
Others: P. Voytek
C. J. Villar
R. R. O'Neill

Research Microbiologist
Visiting IPA Agreement
Guest Worker
Staff Fellow

LMM, NIAID
LMM, NIAID
LMM, NIAID
LMM, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

4

PROFESSIONAL

3

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

Analysis of the genetic basis of virus induced neoplastic disease has led to the identification of many chromosomal genes involved in these processes. Although most of these studies rely on use of the various inbred strains, we have begun to analyze a number of wild mice for infectious MuLVs and for susceptibility to virus induced disease. We have identified a novel infectious MuLV, termed HoMuLV, found in the European wild mouse M. hortulanus. HoMuLV has an ecotropic host range and induces neoplastic disease in NIH Swiss mice. HoMuLV was molecularly cloned and sequenced, and its gp70 and several gag proteins were shown to differ substantially from those of the known ecotropic MuLVs. In another series of experiments, we examined various wild derived mice which contained few copies of a single type of germline provirus for susceptibility to virus induced disease. In particular, M. spretus does not produce infectious virus nor does it express detectable levels of retrovirus-related RNA. However, this mouse produces infectious MCF recombinant virus 5 weeks after inoculation with MCF MuLV and develops lymphoma within 16 weeks. Thus, this mouse contains the endogenous sequences which can contribute to the generation of leukemogenic MuLVs. One germline spretus provirus has been molecularly cloned and is being characterized.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-AI-0301-07-LMM

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular Genes Involved in Transformation and MuLV Transmission in Inbred Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	C. A. Kozak	Research Microbiologist	LMM, NIAID
Others:	M. D. Hoggan	Senior Scientist	LMM, NIAID
	F. Gonzalez	NCI	
	E. Appella	NCI	
	G. Vande Woyde,	FCRC	

COOPERATING UNITS (if any)

M. Crumpton, ICRF, London, England; H. Orr, U. Minnesota; L. Old, Sloan Kettering; J. Gusella, Harvard Med. Sch., Boston, MA

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

4

PROFESSIONAL:

3

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Numerous chromosomal genes have been identified which are involved in retrovirus-induced disease and the transmission of retroviral genes in the inbred mouse. Genetic mapping of these genes to specific mouse chromosomes has been accomplished by the analysis of Chinese hamster x mouse somatic cell hybrids each of which contains different mouse chromosomes. In some cases, genes have also been mapped to subchromosomal regions or to specific map locations along these chromosomes by use of classical Mendelian crosses. These studies have led to the chromosomal mapping of genes encoding the tumor rejection antigen gp96, the *Fms* and *Met* proto-oncogenes and a sequence which may represent the ecotropic MuLV cell surface receptor. The somatic cell hybrids have also been used to map other chromosomal genes for which cloned probes are available. Most recently, we have mapped genes for spermatid associated proteins, genes related to *Drosophila* heat shock proteins, P450 cytochromes, and several T lymphocyte antigens.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI-00304-07-LMM

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

Pathogenesis of Disease Induced by Friend MuLV

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator; Name, title, laboratory, and institute affiliation)

PI: Jonathan Silver

Senior Investigator

LMM, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

1.5

PROFESSIONAL

0.5

OTHER:

1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

As part of our study of proviral integration sites, we have developed a novel application of the polymerase chain reaction (PCR) technique which allows us to amplify small segments of DNA adjacent to a region of known sequence. The basic idea is to cut the DNA with a frequent cutting restriction enzyme such as Sau3A and then to circularize these fragments. Oligonucleotides complementary to opposite strands of DNA from the region of known sequence and pointing 5'→3' away from each other in the original configuration of the linear DNA are then used to amplify the portion of the Sau3A circle which contains the region of unknown sequence. In our case, we are using this method to clone cellular DNA flanking integrated proviruses, but the method promises to be widely applicable to other situations in which one would like to clone DNA adjacent to a previously identified region, such as in "walking" or "jumping" along a chromosome, or in cloning the control region upstream of an mRNA (cDNA) start site.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI-0353-06-LMM
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structural and Functional Studies of Mammalian Endogenous Retroviral Sequences (Revised Title)		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	Arifa S. Khan	Res. Microbiologist LMM, NIAID
Others:	Elisa Vicenzi	Visiting Assoc. LMM, NIAID
	Lisa H. Preis	Biologist LMM, NIAID
	James R. Flanagan	Medical Staff Fellow LIG, NIAID
	Arthur M. Krieg	Medical Staff Fellow ARB, NIAMSD
	Claude F. Garon	Chief LPB, NIAID
	Richard G. Cuttler	Research Chemist IRP, NIA
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Biochemical Virology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.1	3.1	1
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Mammalian genomes contain multiple copies of retroviral-related sequences. The majority of these are defective for virus expression; however, some can produce subgenomic RNAs. Age-dependent expression of endogenous retroviral sequences was studied in <u>Mus musculus</u> wild mouse and man using endogenous MuLV and human 4-1 DNA probes. In both cases transcripts were detected in kidney, brain, and liver tissues, but expression was highest in the kidney. MuLV-related RNAs, 8.4 kb, 3.0 kb, and 1.8 kb were seen throughout life (18 day old embryo - 28 months of age) and increased with age. Transcripts, 3.0 kb and 1.8 kb, were seen in human tissues from 8 month old fetus -85 years of age. These RNAs also appeared to increase with age. The results suggest a relaxation of gene control in mammalian cells with aging. A novel 5.0 kb retrotransposon-like element, B-13, was cloned from BALB/c mouse embryo DNA and sequenced. It was distantly related to known MuLV DNAs: <75% homology was seen in the <u>gag</u> and <u>pol</u> regions; <u>env</u> sequences were absent. Two other cloned DNAs isolated from the BALB/c mouse genome contained B-13-like genes but different flanking cellular sequences. Several copies of B-13-related genes were detected in inbred and closely related wild mouse genomes. These results indicate that B-13-like elements are amplified and dispersed in the mouse genome. Two novel protein binding sites were identified at the 5' end of all MuLV-related LTRs using 30 bp oligomers as probes in gel mobility shift assays. These highly conserved sites were present upstream of the enhancer in a region designated as the upstream conserved region (UCR) and bound nuclear factors designated as UCRF-L and UCRF-U. UCRF-L was the prominent species in murine tissues whereas UCRF-U predominated in cell lines. Northern analysis of 5 different mouse strains indicated a good inverse correlation between MuLV RNA expression and UCRF-L activity. This relationship was seen in case of both spontaneous and LPS-induced MuLV RNAs. These results suggest a negative regulatory function of UCRF-L in MuLV gene expression.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00388-04:LMM

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Functional Analysis of Human Retroviral Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Rabson	Senior Staff Fellow	LMM, NIAID
Others:	W. Maury	IRTA	LMM, NIAID
	B. Potts	Senior Staff Fellow	LMM, NIAID
	M. A. Martin	Chief	LMM, NIAID
	T. Folks	Expert	LIR, NIAID
	K. Clouse	Assistant Professor	Dept. of Microbiology Georgetown University

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

Project Terminated

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00395-04-LMM

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic and Molecular Analysis of Anerobic Bacteria Indigenious to Humans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: C. J. Smith

Sr. Staff Fellow

LMM, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Bacterial Virulence Section

INSTITUTE AND LOCATION

NIAID, NIH, Frederick, MD 21701

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project Terminated

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00415-04-LMM

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

The Molecular Biology of Retroviruses Associated with AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Malcolm A. Martin

Chief

LMM, NIAID

Others: Klaus Strebel

Visiting Associate

LMM, NIAID

Thomas Klimkait

Guest Researcher

LMM, NIAID

Ronald Willey

Biologist

LMM, NIAID

COOPERATING UNITS (if any)

Thomas Folks, and H. Clifford Lane, LIR, NIAID; Richard Klausner and Juan Bonifacino, CBMB, NICHHD; Laurence Laskey, Genentech.

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS

5.0

PROFESSIONAL

2.5

OTHER

2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Site-specific mutagenesis was used to introduce amino acid substitutions at the asparagine codons of four conserved N-linked glycosylation sites within the gp120 envelope protein of human immunodeficiency virus (HIV). One of these alterations resulted in the production of noninfectious virus particles. The amino acid substitution did not interfere with the synthesis, processing or stability of the env gene polypeptides gp120 and gp41 or the binding of gp120 to its cellular receptor, the CD4 molecule. Vaccinia virus recombinants containing wild-type or mutant HIV env genes readily induced syncytia in CD4 positive HeLa cells. These findings indicate that alterations affecting the second conserved domain of the HIV gp120 interfere with an essential early step in the virus replication cycle other than binding to the CD4 receptor.

A 16 kilodalton protein expressed in HIV-1 producing cells was identified as the gene product of the "U" open reading frame (ORF). When expressed in vitro, this 81 amino acid protein (vpu) reacted with about one-third of the serum samples obtained from HIV infected individuals, indicating that vpu is expressed in vivo as well. Introduction of a frame-shift mutation into the vpu ORF did not significantly interfere with the synthesis of the major viral proteins in a transient expression system. However, a five to ten-fold reduction in progeny virions was observed after infection of T-lymphocytes with the mutant virus. These data suggest that the vpu protein is required for efficient virus replication and may have a role in assembly or maturation of progeny virions.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00433-03-LMM

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Use of Retroviruses as TAgged Insertional Mutagens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Jonathan Silver

Medical Officer

LMM, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Viral Biology Section

INSTITUTE AND LOCATION

NIAID, Bethesda, MD 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project Terminated

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI-00434-03 LMM

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on the Dissemination of Streptococcal Antibiotic Resistance Determinants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)

PI: D. J. LeBlanc

Head, BVS

LMM, NIAID

Others: L. N. Lee

Chemist

LMM, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Bacterial Virulence Section

INSTITUTE AND LOCATION

NIAID, NIH, Frederick, MD 21701-1013

TOTAL MAN-YEARS

PROFESSIONAL

OTHER:

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Project Terminated

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI-00437-04-LMM
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Biology and Genetics of the AIDS Retrovirus		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI: M. D. Hoggan Others: K. Straubel T. Klimkait M. Martin T. Kindt H. Kulaga	Senior Investigator Guest Researcher Guest Researcher Lab Chief Lab Chief Staff Fellow	LMM, NIAID LMM, NIAID LMM, NIAID LMM, NIAID LIG, NIAID LIG, NIAID
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 2	PROFESSIONAL: 1	OTHER: 1
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The study of Morphogenesis of HIV particles in various host cells with various isolates and mutant virus strains continues to provide an interesting insight into HIV infection and remains an important tool in correlating biological changes with genetic variation. U gene mutants of HIV demonstrate considerable differences from wild-type virus when examined at 7 days with many cells showing intracytoplasmic immature particles while WT virus shows a few cells with typical mature particles budding off the cell membrane. By day 10, U mutant virus is seen budding from many cells but only a rare mature form is seen. HIV has been shown to replicate in rabbit cells with the production of RT and typical mature particles budding from the cell surface. Several molecular clones of different HIV strains have shown different plaque morphologies when grown in MT-4 cells while little or no difference can be seen when observed in the EM after growth in A3.01, MT-4 or PBL cell cultures. Both rabbits and guinea pigs have been used to raise antibodies against various molecular clones of HIV which can be demonstrated by Western blot analysis. The MT-4 Plaque test has been used to demonstrate recombination between different HIV mutants with defects in different genes.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI-00438-04-LMM

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Molecular Biology of Cellular Oncogenes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Eugenio Santos	Visiting Scientist	LMM, NIAID
Others:	Theodore Bryan	Microbiologist	LMM, NIAID
	Mercedes Tamame	Guest Researcher	LMM, NIAID
	Teresa Alonso	Guest Researcher	LMM, NIAID
	Angel R. Nebreda	Visiting Fellow	LMM, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

5

PROFESSIONAL

5

OTHER:

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|--------------------------------------|
| ☐ (a) Human subjects | ☐ (b) Human tissues | ☐ (c) Neither |
| ☐ (a1) Minors | | |
| ☐ (a2) Interviews | | |

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

p21 ras proteins and cellular signal transduction systems. - ras genes belong to a multigene family showing high degree of conservation which suggests that they have essential cellular functions. The similarities of ras proteins with the class of guanine-nucleotide binding (G) proteins support the notion that ras gene products participate in internalization of extracellular signals.

However, ras proteins are not G-type regulatory components of mammalian adenylate cyclase. In addition we recently showed that they are neither direct regulatory elements of phospholipase C nor of phospholipase A2 in inositol-lipid signalling pathways. Furthermore, by using radiation inactivation, we have also demonstrated that ras proteins have an homooligomeric structure which does not conform to the structure of monomeric alpha subunits in classical G proteins ($\alpha\beta\gamma$ heterotrimer).

Cascades of phosphorylation may constitute alternative efficient systems to transmit cellular signals. We have recently found that meiotic maturation of Xenopus oocytes induced by microinjected ras proteins is associated with phosphorylation of specific cellular substrates. These observations indicate that ras proteins participate in signal transduction by triggering phosphorylation of specific cellular substrates and suggest that these proteins may regulate cascades of phosphorylation involved in control of the cell cycle.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00464-02-LMM
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Plasmid Replication and Expression in Streptococci		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: J. M. Ranhand Senior Scientist LMM, NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Bacterial Virulence Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☐ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Project Terminated		

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00466-03-LMM

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT: 80 characters or less. Title must fit on one line between the borders.

Transcriptional Complexity of AIDS Retrovirus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)

PI: Sundararajan Venkatesan

Expert

LMM, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemistry Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

PROFESSIONAL

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project Terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI-00467-03-LMM
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structure and Function of Polypeptides Encoded by AIDS RV Genome		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI: Sundararajan Venkatesan Other: Nafees Ahmad Robert J. Mervis Eric Lillehoj	Expert Visiting Fellow Microbiologist Scientist Emeritus	LMM, NIAID LMM, NIAID LMM, NIAID FCRC, NIAID
COOPERATING UNITS (if any) Michael G. Raum, Chemist, BRB, NIAID; Hardy Chan, Res. Director, SYNTEX Corp.; F. H. Rick Salazar, Microbiologist, SYNTEX Corp.		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Biochemical Virology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 3.5	PROFESSIONAL: 3	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The genome of the human immunodeficiency virus (HIV), the etiological agent of AIDS, codes for several polypeptides by means of an extremely complex scheme of mRNA biogenesis. Using defined antisera, we have purified and sequenced pol ORF encoded proteins, both from extracellular virus and cellular lysates. Previously, we identified and sequenced the p64 and p51 RT subunits of gag and pol ORF as well as the p34, the putative integration protein, mapping near the C-terminus of the pol RT. We have extended this project and sequenced a p10 gag-pol protease responsible for the processing of the gag and gag-pol fusion protein. A protein of similar size and identical N-terminal sequence (determined through the first 39 residues) was present among the processed HIV polgene products in <i>E. coli</i> expressing the entire HIV pol ORF. Although the purified viral protease lacked measurable proteolytic activity, the bacterial extracts were capable of processing an HIV gag precursor protein synthesized in <i>E. coli</i>. We have also developed a sensitive assay for the protease using defined oligopeptides and this will enable us to screen several protease inhibitors as potential antiviral drugs.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1AI-00526-01-LMM

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

To Investigate the Effects of the Expression of HIV Envelope Gene in CD4+ Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Jonathan Silver
Other: James Felser
Thomas Klimkait

Senior Investigator
Medical Staff Fellow
Guest Researcher

LMM, NIAID
LMM, NIAID
LMM, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Viral Biology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

1.5

PROFESSIONAL

1.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To facilitate studies of the effects of expression of HIV envelope proteins, we made an expression vector by deleting segments of gag and pol genes from the infectious HIV provirus clone pNL4-3. Following transfection of this clone into a variety of human cell lines, HIV envelope proteins are made in amounts comparable to those made after transfection of wild-type HIV proviral DNA. A syncytia assay was developed which detects HIV envelope proteins following transfection of HIV envelope-expressing plasmid DNAs into a modified HeLa cell line which expresses on its surface the HIV receptor molecule CD4. Using this assay, we found that mutations in two portions of the HIV env gene ablate syncytia formation: a region of the env gene which is necessary for binding to CD4, and a hydrophobic region at the amino terminus of the gp41 env peptide. We plan to use these envelope mutants to study the effects of long term HIV envelope expression in CD4 positive cells, with particular interest in how the HIV envelope affects expression of CD4 and infectibility by HIV.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI-00527-01-LMM
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Cloning and Characterization of Retroviruses Associated with AIDS		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Theodore S. Theodore	Research Microbiologist LMM, NIAID
Others:	Malcolm A. Martin	Chief LMM, NIAID
COOPERATING UNITS (if any) Thomas Folks, Expert, LIR, NIAID		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Biochemical Virology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1	PROFESSIONAL: 1	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Heterogeneity among AIDS retroviral genomes has been clearly demonstrated. We have used molecular cloning, restriction enzyme analysis, and nucleotide sequencing techniques to characterize isolates of the Human Immunodeficiency Virus (HIV). These studies may provide data regarding their structural as well as geographic diversity, and possibly the degree of antigenic variation and pathogenicity among different HIV isolates. Since African isolates as a group are more diverse than those of North America and Europe, we molecularly cloned an African (Zaire) HIV isolate. This clone contained a single LTR (9.2 KB genome) and upon transfection into lymphocytes, infectious virus particles were produced after 14 days. Reverse Transcriptase (RT) activity resembled that of the constructed infectious clone (PNL 4-3), and upon passage onto fresh lymphocytes, displayed similar RT kinetics as PNL 4-3. The complete nucleotide sequence of this infectious molecular clone is now being analyzed and attempts to prepare antisera to this African isolate for neutralization tests are in progress.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00528-01-LMM

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Functional Interactions of HIV Regulatory Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Sundararajan Venkatesan

Expert

LMM, NIAID

Others: Nafees Ahmad
Robert J. MervisVisiting Fellow
MicrobiologistLMM, NIAID
LMM, NIAID

COOPERATING UNITS (if any)

LAB BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemistry Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

2.5

PROFESSIONAL

2

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The trans-complementation of a replication defective human immunodeficiency, type 1 (HIV-1), art/trs negative mutant provirus by the art/trs protein was characterized by amplified viral replication. The enhanced trans-activation was unique to the art/trs mutant, whose phenotype was distinguished by accumulation of HIV-1 B(3')-ORF and tr proteins and virtual lack of viral structural protein synthesis. The virus produced by trans-complementation was not infectious for CD4+ lymphocytes and the rescued phenotype could not be passaged by cocultivation of transfectants with CD4+ lymphocytes thus eliminating the possibility that the mutation was rescued by recombination. The magnitude of transactivation of mutant provirus was relatively constant over a wide range of art/trs expressing cDNA (0.5-20 μ g). art/trs protein was also absolutely required for the transient expression of env gp160 from subgenomic plasmids containing HIV env ORF (in the spliced or presliced configuration) fused to either HIV LTR or an heterologous promoter.

The 3'(B)-ORF product of the Human Immunodeficiency Virus type 1 (HIV-1) was shown to be a negative regulator of HIV-1 replication. B ORF mutant proviruses replicated more vigorously than their standard counterparts during transient expression in a variety of nonlymphoid and lymphoid cell lines. The B ORF product supplied in trans as an expressible cDNA, suppressed in a dose-dependent manner, the standard replication of wild type provirus and the enhanced replication of B ORF mutant proviruses. Transient expression of reporter genes that were linked to the HIV LTR was also repressed by coexpression of B ORF. The negative effect of B ORF was mediated by a concentration dependent inhibition of transcription from HIV-1 LTR containing a to be negative regulatory element, located between 340 and 156 nucleotides upstream of the RNA initiation site.

LABORATORY OF PARASITIC DISEASES
1988 Annual Report
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ADMINISTRATIVE AND ORGANIZATIONAL EVENTS

As this report is being prepared we are bracing for the big move to renovated facilities in Building 4. Undoubtedly there will be some complaints and disappointments, but we are grateful for the efforts by everyone who has labored so hard to make the move possible.

Professional personnel who left this fiscal year include: Dr. Russell Howard, a Visiting Scientist who had been approved for tenure at NIH, and who had earned a position of research leadership in LPD, resigned to take a position and join his wife at DNAX in Palo Alto, California. Medical Staff Fellows Richard Davey has taken a position with LIR for clinical work on AIDS; Rodney Adam takes up a faculty position in Infectious Diseases at Univ. of Arizona; Steve Puentes will be on the faculty at Univ. of California Los Angeles. Visiting Associate Paul Bates returned to the U.K. to a position at the Univ. of Glasgow, and Guest Researcher Maurice Odera returns to his job in Kenya. We were saddened and will greatly miss long-time LPD research assistant Elwood Claggett who succumbed to a chronic illness.

There has been considerable turnover in secretarial staff in the last year: Wilma Davis left to take another position in Bldg. 31; Linda Sacchetti moved into an administrative job with NIAID Intramural; and Jeanne Carolan took a Lab Chief's secretarial position in NCI. After a long period of recruiting difficulties, we finally seem to be settled with excellent help from Gerri Carter as Chief's secretary, Sheryl Rathke as Editorial Assistant, Tracy Venison and temporaries Laurie Dixon and Veronique Rynn.

New personnel coming to LPD include the following: Two Medical Staff Fellows Amy Klion and Bach-Yen Nguyen, the latter to spend most of her first year on the wards in LCI. Two other Medical Staff Fellows who came to LPD from LCI are Chris King and Steve Dolin. David Peterson is an IRTA Fellow and John Adams a Staff Fellow, both of whom join the Malaria Section of LPD. Klaus Sieber is another new Staff Fellow in the Malaria Section. Guest Researchers on short term appointments include Alok and Sudha Bhattacharya from India, Rabia Hussain from Pakistan, and John Dalton from Ireland.

During the past year Dr. Keith Joiner and his group in LCI were transferred administratively to LPD as a Unit on Microbial Pathogenesis. Much of the research work of this group is focused upon the role of serum complement in reacting with parasites and in parasite interactions with host cells. There has been and continues to be a good deal of research collaboration between Dr. Joiner's group and LPD staff.

The travel of LPD staff to scientific meetings abroad will not be listed here. However, as usual, there was considerable foreign travel by members of LPD to carry out research work. Dr. Ottesen went to Madras, India, to help carry out treatment trials of lymphatic filariasis with Ivermectin. Dr. Nutman and Freedman worked on onchocerciasis in Guatemala. Dr. Cheever spent several weeks in the Sudan participating in the Univ. of Michigan State's schistosomiasis project there. Dr. Melby worked in Patna, India, on leishmaniasis. Dr. Gwadz went to Mali, West Africa, to investigate possibilities for LPD to work on malaria vectors there; he also was in Egypt and Israel as an advisor to their cooperative research project. Dr. Ballard went to Honduras for collection of research specimens and Dr. Neva also went to Honduras for work on leishmaniasis.

HONORS AND AWARDS:

In addition to service on NIH or NIAID committees, which seem to require more and more staff time, many of the professional staff serve on WHO and/or TDR Expert Committees, or on Ad Hoc Committees for various granting agencies or foundations. They are also invited to participate in meetings or workshops and spend a great deal of time reviewing manuscripts. Such recognition and honors are too numerous to be individually cited.

However, specific mention is warranted for the following items of special recognition or honor:

Dr. Allen Cheever was the recipient of the Outstanding Service Medal of the USPHS.

Dr. Louis Miller was an invited speaker for celebration of the 100th anniversary of the Pasteur Institute in Paris and also again an invited speaker to the English Medical Research Council Seminar Series -- both in October 1987.

Dr. Miller was elected next President of the American Society of Tropical Medicine and Hygiene.

Dr. Thomas McCutchan was promoted from GS-13 to GS-14 Microbiologist.

Research Progress

IMMUNOREGULATION IN FILARIAL INFECTIONS

Similar mechanism found for high IgE in filariasis and a non-parasitic hyper-IgE syndrome. Extremely high serum IgE levels are characteristic of certain filarial disease, but also are seen in some non-parasitic diseases such as Job's syndrome. As

reported last year, T-cell clones derived from these patients were found to be making large amounts of IgE binding factors. But one of these clones was also found to produce large amounts of IL-4, along with a B cell growth factor. Since T cell products, such as IL-4 and γ interferon, are likely to regulate IgE response, INF- γ was found to down regulate the IgE response in patients with hyper-IgE states. The effect was T-cell independent and isotype specific. By contrast, IL-4 appears to have an opposite effect on human lymphocytes, causing a marked enhancement of the IgE response. Preliminary results suggest that regulation of serum IgG4 levels is similar to that of IgE (Nutman and Gallin of NIAID and Delepesse of Univ. of Montreal).

Definition of humoral and cellular subclasses in filariasis and onchocerciasis. The various clinical manifestations of filariasis, which are known to reflect different pathways of immune response, are also characterized by differences in IgG subclass responses to the parasite. In comparing elephantiasis patients vs. those with microfilaremia, IgG1 and IgG2 responses to adult worm antigens by immunoblotting were comparable. However, IgG3 responses to numerous parasite antigens were prominent in the elephantiasis group but essentially non-existent in the microfilaremics. Conversely, IgG4 responses were very prominent in the microfilaremics but virtually absent in those with elephantiasis (Ottesen, Nutman, and Hussain of LPD and Kumaraswami of India).

Immunologic unresponsiveness in filariasis patients. From previous work it is well known that ceratin patients with filariasis (microfilaremics) have T-cell anergy, while others (chronic elephantiasis and occult filariasis) have good T-cell responses to crude filarial worm antigens. Similar T-cell unresponsiveness was found in a group of patients with active onchocerciasis. Using a model of in-vitro antibody production, this failure of specific immune response was also shown to exist at the B-cell level. Since some of the anergic patients have circulating antigens containing phosphocholine (PC) determinants, the immunosuppressive properties of PC are being investigated (Nutman and Ottesen).

IMMUNODIAGNOSIS, CHEMOPROPHYLLAXIS AND TREATMENT OF FILARIASIS

Molecular definition of filarial antigens. Because crude filarial antigens are often cross reactive with other helminth parasites, and antigens from specific stages of the parasite may be limited in amount, efforts have turned to obtain specific parasite antigens from cloned gene products. For example, one

clone, a 125 kD fusion protein, obtained from crude onchocerca adult worm antigen, appears to distinguish between sera from immune individuals vs. those infected with the parasite. A cDNA library has been prepared from *W. bancrofti* microfilaria, and a clone from *B. malayi* has been shown to stimulate T-cells of infected patients; this latter clone has been fully sequenced and peptides with putative T cell recognition sites are being sequenced (Nutman, Raghavan, Colina and Ottesen of LPD with Rajan and Arasu of New England Biolabs). By Western blotting sera of patients with onchocerciasis recognize a 12 kD antigen that seems to be specific and a 22 kD antigen is preferentially seen by loiasis patients (Nutman and Ottesen of LPD with collaborators in Madras, India and Guatemala). A 43 kD antigen derived from infective larval (L3) *B. malayi* was found to show promising specificity in Western blots in identifying protective antibody against *W. bancrofti*. Because of the scarcity of L3 material from *B. malayi*, and the complete unavailability of L3s from *W. bancrofti*, the epitope or epitopes on the 43 kD antigen will have to be amplified by recombinant techniques (Freedman, Nutman and Ottesen).

Diethylcarbamazine found to prevent Loa loa in Peace Corps volunteers. The double-blinded, placebo-controlled trial of diethylcarbamazine (DEC) prophylaxis (300 mg weekly) in Peace Corps volunteers in 3 West African countries was concluded. Exposure to the parasite turned out to be heaviest in Gabon where 6 of 20 (30%) individuals in the placebo group developed overt clinical disease after a two year tour of duty, vs. 0 of 16 in the DEC treated group. A higher seroconversion rate (10 of 20 or 50%) in the placebo group as compared to the DEC group (2 of 16 or 13%) was also found. Side effects attributable to DEC were minimal. These findings indicate that DEC given orally once weekly can be an effective and acceptable chemoprophylactic for prevention of loiasis in individuals who require an extended stay in Loa loa endemic areas of Africa (Ottesen and Nutman of LCI, Miller and Mulligan of Peace Corps).

Ivermectin treatment of lymphatic filariasis. Ivermectin, a newly developed drug with effectiveness for animal nematodes and ectoparasites as well as human onchocerciasis, was assessed for its activity in W. bancrofti human filariasis in collaboration with Indian scientists in Madras, India. Two phase 2 trials were carried out, the second one comparing Ivermectin at two doses (20 and 120 mcg/kg once orally) vs. a standard two-week course of DEC (at 6 mg/kg/day). The results showed comparable efficacy of both Ivermectin dosages and DEC after 3 months, but by 6 months the microfilaria recurrence rate of both Ivermectin groups was significantly greater than in the DEC group. Although side effects were approximately equal in the DEC and Ivermectin groups, the single oral dose of Ivermectin with no greater toxicity than DEC would undoubtedly have greater compliance for the former drug (Ottesen and Nutman of LPD, M. Aziz of Merck Sharpe & Dohme and Kumaraswami in Madras, India).

MOLECULAR BIOLOGY OF GIARDIA & AMEBAE

More on the variant surface antigen and DNA characteristics of Giardia. The phenomenon of antigenic variation in Giardia has now been demonstrated in several different isolates and can involve more than one surface antigen, although the 170 kD antigen has received the most study. While cytotoxic poly or monoclonal antibodies against the 170 kD antigen are generally used to produce progeny without this marker, the loss and acquisition of new variant antigens also occurs without such selection pressure -- i.e., by passage in culture. Analysis of parent-progeny pairs with molecular probes to 170 kD suggests that the antigenic variation is due to some type of genomic rearrangement (Adam, Nash, Aggarwal, McCutchan and others in LPD). A constant pattern of chromosomal banding (4 or 6 major bands) was found in 6 Giardia isolates using pulse field gradient electrophoresis, but minor bands thought to represent linear fragments of DNA are also seen (Adam, Wellem, Nash). In the course of transfection experiments of Giardia with a Drosophila and heat shock protein genes, certain isolates of the parasite were found to lack the latter marker (Aggarwal, Romans, de la Cruz and Nash).

What happens to the variant antigen in-vivo? Since a rather confusing pattern of self-cure and resistance to challenge was found in gerbils infected with Giardia the relationship of these findings to antigenic variation was investigated. The major 150 kD antigen was lost within 7 days and replaced by a series of other antigens which then remained unchanged (Aggarwal and Nash). Three separate experiments infecting human volunteers with Giardia have been conducted. One parasite clone was found to be non-infective. Variation in the 60 kD antigen also occurred in humans; however, no cyclical or predictable pattern of antigenic changes could be identified (Nash of LPD, Herrington, Levine and Lyonsky of Univ. of Maryland).

Studies with amebae. Highly repeated patterns of total genomic DNA were found in different species of amebae. However, clones of ribosomal DNA proved to be useful hybridization probes to distinguish between different species of amebae. A rather

surprising finding is the presence of circular ribosomal DNA in *E. histolytica* (S. Bhattacharya & Diamond). A series of monoclonal antibodies have been generated against *E. histolytica* antigens (A. Bhattacharya and Diamond). Further evidence has been obtained to confirm the previous finding that non-pathogenic strains of *E. histolytica* change their isoenzyme pattern when the organisms are axenized (Diamond in collaboration with Mirelman of Israel and Jalan of India).

MOLECULAR BIOLOGY OF MALARIA VACCINES

Gene for ookinete surface antigen of *P. falciparum* cloned.

One of the malaria vaccine strategies pursued by the LPD group was the concept of a vaccine that would interfere with the sexual stage of parasite development in the mosquito and thus block transmission. After long efforts and many technical difficulties, a 25 kD antigen on the surface of ookinetes of *P. falciparum* has been sequenced and the gene cloned. The protein is rich in cysteine, has some structural similarities to human epidermal growth factor and low density lipoprotein receptor, and probably does not have capacity for antigenic variation. Although much work remains, the 25 kD antigen appears to be a very good vaccine candidate (Kaslow, Quakyi, Syin, Miller and Coligan of NIAID; Houghton at Scripps and Laskey at Genentech). Two other surface antigens, 230 and 48/45 kD on *P. falciparum* gametes and zygotes are also under investigation. Because of limited material to work with, an indirect approach using cross-hybridization with synthetic oligonucleotides to probe *P. gallinaceum* RNA is being used. Cross hybridization with the 25 kD protein has been demonstrated, and it is hoped that the same approach will work with the 230 and 48/45 kD proteins (Syin, Quakyi, Kaslow, Miller, McCutchan of LPD and Coligan and Raum of BRB).

A unique 66 kD merozoite surface antigen from *P. knowlesi*. This antigen is of particular interest because it is synthesized in mature schizonts just before merozoite release, makes up only a tiny fraction of total merozoite protein, and yet appears to be critical in the invasion process of red cells. cDNA clones for the gene have been isolated and sequenced. It is likely that a homologous gene is present in the human parasite, *P. vivax* (Waters and McCutchan).

IMMUNOLOGY OF MALARIA VACCINES

Evidence that cytotoxic T cells are important in immunity to sporozoites.

Mice immunized with *P. yoelli* sporozoites were depleted of CD4⁺ (helper) and CD8⁺ (suppressor and cytotoxic) cells by administration of monoclonal antibodies against these surface molecules. Immune mice depleted of their CD4 T cells were still fully immune on challenge with infectious sporozoites, while those depleted of CD8 T cells were not protected. It was concluded that the CD8 cells function through a cytotoxic mechanism (Weiss, Good, Berzofsky, Kumar and Miller of NIH, Beaudoin and Sedegah of NMRI and Holligdale of BRI). In order to identify epitopes of the circumsporozoite protein (CS), which were targets for cytotoxic T lymphocytes (CTL), murine L cells were transfected with the gene for CS protein of *P. falciparum*. By IFA the transfected cells were shown to express CS protein in the cytoplasm, but not on the cell surface. To generate CS specific CTLs mice were either inoculated with a recombinant vaccinia virus containing the CS gene, or were immunized with sporozoites. By appropriate cytotoxicity tests it was shown that specific killing occurred only in the L cells transfected with the CS gene. Further, by testing target cells of different H-2 background it was shown that the CTL response was genetically restricted. The implication of these experiments is that *P. falciparum* CS protein can serve as the epitope for CTL without being expressed on the cell surface. (Kumar, Good, Miller of LPD and Berzofsky of NCI).

Evolution of malarial CS protein gene and implications for a sporozoite vaccine. If a sporozoite vaccine for malaria is to be effective, the target CS protein should be

stable and not subject to antigenic variation. Analysis of genes for the CS protein from many species and isolates of malaria parasites, however, suggests that the central repeat domains of the gene are evolving. Also, there is evidence that non-repetitive regions of the gene are under intense selective pressure, presumably immunologic, leading to mutations that would affect T cell activation (de la Cruz, McCutchan and Lal of LPD and Campbell and Procell of CDC).

MOLECULAR GENETICS OF MALARIA PARASITES

Chromosome mapping of *P. falciparum*. By pulse field gradient electrophoresis, 14 chromosomes, ranging in size from 800 to 3500 kb, have been identified on *P. falciparum*. Chromosomal DNA is cut by restriction enzymes at only 1 to 5 sites per chromosome so chromosome mapping is facilitated. Chromosome 4 carries a linkage group controlling pyrimethamine susceptibility (Wellems). Genetics of drug resistance in *P. falciparum*. A clone of *P. falciparum* sensitive to various antimalarial drugs was crossed with a multi-drug resistant clone. This was done by growing the parasites *in vitro* to produce gametocytes, mixing the gametocytes to feed mosquitoes and thereby achieving true genetic mixing, allowing the infected mosquitoes to bite and infect a chimpanzee and then recovering multiple clones of asexual progeny from the blood for genetic analysis. The gene encoding dihydrofolate reductase-thymidylate synthetase (DHFR-TS) was found to be near the central region of chromosome 4. It was concluded that a mutation within the DHFR-TS gene was responsible for pyrimethamine resistance. By analyzing the frequency of resistance to different drugs in multiple clones it was concluded that chloroquine and mefloquine resistance are inherited independently -- i.e., under control of different genetic loci. No evidence was found for gene amplification. A sufficient number of progeny clones have been recovered to identify the genetic locus governing chloroquine resistance (Wellems, Panton, Peterson of LPD and Rosario of BRI and Morry and Cross of Rockefeller University).

Is asexual proliferation of *P. falciparum* under genetic control? The parent clones of *P. falciparum* used in the genetic cross experiment (3D7 and HB3) were found to have different proliferation rates when grown *in vitro* in human RBCs. Clone 3D7 grew nearly twice as fast as HB3, and when mixed together, clone 3D7 completely overgrew HB3 by 10 days. The difference in growth rates is not due to numbers of merozoites produced or the maturation time of the parasite; it appears to be due to efficiency of re-invasion of host red cells. By linkage group analysis the rapid growth rate in 3D7 was found to associate with a genetic locus on chromosome 13. Since proliferation rate of *P. falciparum* could be an important factor in virulence of a parasite strain the mechanism for this genetic trait is being investigated (Wellems and Panton of LPD and Rosario of BRI).

MOLECULAR BASIS OF MALARIA PARASITE INVASION OF RBCs

A 135 kD protein appears to identify the Duffy red cell receptor. Human RBCs that lack the Duffy blood group antigens (a and b) are refractory to infection by *P. knowlesi* *in vitro* and *P. vivax* *in vivo*. A 135 kD protein found in culture supernates of *P. knowlesi* was found to bind strongly to Duffy b cells, weakly to Duffy a cells and did not bind to Duffy negative cells. Certain sugars and sulfated polysaccharides that block binding of this parasite protein also block the attachment of merozoites and invasion of RBCs. It is hoped that antibodies to the 135 kD protein will identify portions of the molecule in a cDNA expression library so it can be cloned (Miller and Dalton of LPD, Hadley and Haynes of WRAIR).

Receptor specificity for *P. falciparum* merozoites is as confusing as ever! There is still a great deal of confusion concerning receptor(s) required for invasion of RBCs by *P. falciparum*. Some parasites require sialic acid on the erythrocyte membrane, but others

invade independent of sialic acid. By using red cells of different types it has been shown that glycoporphins A, B or C are not necessary for invasion. Whatever ligand on the RBC surface is required is trypsin sensitive. A recent surprise is the finding that red cells of certain strains of mice, but no other rodents, are susceptible to invasion by P. falciparum (Miller and Klotz of LPD, Hadley and Haynes of WRAIR).

RIBOSOMAL RNA OF MALARIA PARASITES Ribosomal RNA genes as markers of Plasmodial development and evolution. Two structurally distinct nuclear genes have been found to encode the ribosomal RNAs of Plasmodium berghei.

One type of gene is found almost exclusively in sexual stages of the parasite found in the mosquito. The second type of ribosomal gene is the predominant ribosomal RNA species for the asexual stage in the mammalian host. This switching off and on of RNA is of particular interest in parasites which go through developmental stages in different hosts. Ribosomal RNA also serves as a useful marker in analyzing the evolutionary relationships of different species of malaria parasites. This approach is yielding an "evolutionary tree" which is quite different from that derived by classical taxonomic criteria. One further advantage of using ribosomal RNA probes is that they offer a more sensitive method in detecting minimal amounts of parasite material -- this could be exploited for diagnostic use (McCutchan).

Altering vector susceptibility to infection with the malaria parasite. Several approaches continue to manipulate susceptibility of A. gambiae to the malaria parasite. Phenoloxidase metabolism appears to be involved in the encapsulation process around ookinetes in the refractory strain. The newly discovered antimicrobial agent, magainin, disrupts oocyst development (Gwadz of LPD and Zaslof of NICHD). Another approach, that of introducing foreign DNA into the germ line of mosquito vectors has benefited from technical improvements in that nearly 25 per cent of eggs injected will hatch. The neomycin resistance gene that was successfully introduced has gone through 6 generations, so the integration event was stable. Other transposon vectors are being tested to increase efficiency of foreign gene integration (Sakai, Miller, Lee, Romans and Gwadz of LPD, Mizuuchi of NIDDKD and Coon of NCI).

IMMUNOLOGY AND IMMUNOPATHOLOGY OF SCHISTOSOMIASIS Schistosome antigens that may be important in immunity. The internal myofibrillar protein from schistosome worms, paramyosin, was compared with another recently described vaccine immunogen, glutathione-S-transferase (GST), reported by French and Australian workers. At equivalent doses, GST was a less potent immunogen than paramyosin, and a combination of the two antigens did not give additive protection (Pearce, James and Sher). In a collaborative project in Brazil, evidence for immunologic reactivity to paramyosin was tested in individuals from a schistosome endemic area. Results suggest that asymptomatic, non-infected, but exposed individuals have high levels of serum antibody to paramyosin. In contrast, hepatosplenic patients appear to have unusually low responses to paramyosin (R. Correa-Oliveira of Brazil and Sher of LPD). A highly T cell reactive antigen fraction has been demonstrated by immunoblotting experiments with lymphocytes from immunized mice. The antigen is present in supernates of adult worms (SWAP), it is not paramyosin, and it can be purified from SWAP by FPLC chromatography (James, Pearce and Sher).

Hepato-splenic schistosomiasis in a Sudanese population assessed by ultrasound. The usefulness of ultrasound examination of the liver to detect hepatosplenic disease in schistosomiasis was confirmed and extended by a prospective study in which the diagnosis was confirmed by wedge biopsy of the liver. All 29 patients among a total of 41 studied who had Symmer's fibrosis by pathological examination were correctly diagnosed by ultrasonography. This technique will be used to assess reversibility of Symmer's fibrosis

after treatment (Nash and Cheever of NIH, Bennett of Michigan State and Homeida of Sudan).

MOLECULAR BIOLOGY OF SCHISTOSOMES

Sequencing the entire paramyosin gene. Although a recombinant paramyosin protein can be produced, it is only about half the native molecule and it induces relatively poor protection as an immunogen. Several approaches are being used to complete the paramyosin sequence. The molecule was shown to be encoded by an unusually large transcript (Knight, Coligan and Sher).

Lipid anchors on schistosomulum membrane proteins. Four major schistosomulum surface antigens were shown to be anchored in the worm tegument by phosphatidylinositol linkages and released in the form of membrane vesicles. Since living schistosomula can stimulate paramyosin-specific T cells, it indicates that paramyosin or some component of it is also released from the worm (Pearce and Sher).

Molecular basis for drug resistance in schistosomes. Although hycanthone is now little used for treatment of schistosomiasis, a chemically related drug, oxamniquine, has been commonly used in Brazil. Resistance of parasites to oxamniquine has been documented, and they are cross-resistant to hycanthone. Drug resistance can be induced by administration of drug to immature worms and raising parasites from eggs recovered later. Restriction fragment length polymorphisms (RFLPs) of characteristic nature can be identified in DNA from drug resistant organisms – even in individual adult worms. It is concluded that drug resistance is the result of a mutation or induction process rather than selection of drug-resistant organisms (Brindley, McCutchan and Sher of LPD, Lewis of BRI and Strand of Johns Hopkins).

LEISHMANIAL BIOCHEMISTRY AND CELL BIOLOGY

A surface lipophosphoglycan (LPG) determines infectivity of leishmania. The differentiation of *L. major* promastigotes from a non-infective to an infective or metacyclic stage is accompanied by changes in surface carbohydrates detected by the lectin, peanut agglutinin (PNA). On non-infective, log phase organisms PNA binds to the surface lipophosphoglycan (LPG). As the organism transforms to the infective stage the LPG is modified so that it no longer binds PNA, and it expresses a new antigenic epitope recognizable by monoclonal antibody. By treatment of the LPGs with phospholipase C, the metacyclic phosphoglycan component of the molecule has been shown to differ in charge and size (Sacks and Joiner at NIH and Turco of Univ. Kentucky).

Surface membrane transporters of leishmania. Three different carrier-mediated transport systems in *L. donovani* promastigotes have now been identified and characterized. One involving ribose functions via facilitated diffusion, but the glucose/fructose and L-proline transport systems are high affinity, active transport systems. An antibody made against anti-proline IgG will be tested to see if an anti-idiotypic reagent will recognize and block functional activity of the proline-transport protein (Dwyer and Pastakia).

Soluble acid phosphatases (SACPs) of leishmanial species compared. SACPs are prominent constituents of all species of *Leishmania* except *L. major*, being secreted by promastigotes and released into the medium. The SACPs of 15 different leishmanial strains, representing 4 species, were found to be antigenically cross-reactive with only slight differences by SDS-PAGE (Doyle and Dwyer).

IMMUNOLOGY OF
LEISHMANIA AND
LEISHMANIAL
INFECTIONS IN MICE

Why are infective leishmania resistant to serum complement?
Since log phase leishmania activate the alternative complement (C) pathway and are rapidly killed, the mechanism by which infective stationary phase organisms resist C killing has been a mystery. It turns out that the infective forms do activate

complement, but do so via the classical pathway. Moreover, the terminal lytic components (C8 and C9) are consumed but rapidly released from the cell membrane, preventing cell lysis. In addition, at least so far as the species *L. major* is concerned, complement and the surface lipophosphoglycan (LPG) referred to previously, play an important role in internalization of the parasite by macrophages. Binding of infective organism to human macrophages is C dependent and involves binding of C3b LPG on the parasite surface. It is postulated that because the membrane attack C complex is released, then macrophage CR1 receptors are able to take up the organism (Sacks, Puentes, da Silva and Joiner).

Protective T cell of mice and relevant leishmanial antigen identified. A soluble antigen fraction 9 from *L. major*, separated by FPLC, was reported last year to confer protection in immunized BALB/c mice. On the basis of protein content fraction 9 is at least 100-fold as effective as the total soluble antigen. Another fraction, 1, was recognized by T cells but failed to protect. T cell lines reactive against the two fractions were established; the line against fraction 9 also protected by adoptive transfer and was found to be subset T_H1. The cell line against fraction 1 was subset T_H2 and actually exacerbated leishmanial lesions. Supernatants from the fraction 1 T cells downregulate macrophage ability to respond to γ interferon. A T cell clone has been made from the line vs. fraction 9 and was found to confer protection equivalent to that obtained with the line. This is the first report of a T cell clone recognized by a defined leishmanial antigen which adoptively transfers protective immunity (Scott, Pearce and Sher of LPD and Coffman of DNAX in California).

IMMUNOLOGY OF
HUMAN
LEISHMANIAL
INFECTIONS

Analysis of T cell responses to 2D immunoblots of leishmanial antigens. Human T cells from patients with cutaneous (CL) or mucocutaneous leishmaniasis (MCL) recognize > 50 distinct antigens separated, blotted and transferred to culture wells. In patients with past or inapparent *L. donovani* infections,

responses were limited to fewer antigens -- especially at lower molecular weights. Interestingly, some examples of dissociated response to antigen fractions were noted; i.e., production of γ -interferon but little or no lymphocyte proliferation. This procedure can also be used to identify antigens recognized by T cell lines and clones, including those recovered directly from leishmanial lesions. It is hoped that cDNA probes for different cytokine mRNAs can be used to assay responses of activated T cells (Melby, Sacks, and Neva).

Diffuse cutaneous leishmaniasis (DCL) in Mexico. In the study referred to last year, all isolates from DCL cases in Mexico were found to be *L. m. mexicana*, as were the isolates in patients with ulcerative, regular cutaneous leishmaniasis (CL). However, the Mexican isolates differ from the same species recovered earlier from Dominican Republic patients with DCL by being more pathogenic for mice. We believe we now have an explanation for our problems in studying lymphocyte blastogenic responses in DCL patients using cryopreserved cells. The cells responsible for failure of lymphocyte proliferation (?suppressor) appear to be sensitive to freezing and are lost. By contrast, we have been able to demonstrate clearly that lymphocyte responses with cryopreserved cells from leishmaniasis cases with normal immune responses are equivalent to responses with fresh cells (Neva of LPD with Kreutzer of Ohio, McMahon-Pratt of Yale, Velasco in Mexico).

Unusual cutaneous leishmaniasis in Honduras. Collaborative studies have been initiated with Dr. Ponce of Honduras of an unusual form of cutaneous leishmaniasis apparently due to L. donovani chagasi, the species of parasite normally causing visceral leishmaniasis in Latin America. At least 16 cases of the cutaneous disease have occurred in an island community of about 8000 inhabitants where similar number of cases of kala azar have occurred over the last 10 years (Neva of LPD, Ponce of Honduras).

**T. CRUZI AND
CHAGAS' DISEASE**

Brown and white clones of T. cruzi have biochemical but no racial implications. In growing clones of T. cruzi it was noted that the pelleted organisms vary in color from dark brown to nearly white. Two clones representing the color extremes were subjected to microelemental image analysis by electron beam X-Rays for various elements, including Fe, Zn and S. Levels of S were equal but the brown clone contained 3 times more Fe than the white clone and only half as much Zn. The only biochemical features that could be associated with these differences were that oxygen consumption was stimulated by glucose in the white clone and that cytochrome levels were much higher in the brown clone. Otherwise, the significance of these differences is not clear (Engel and Dvorak).

Anti-trypansomal factor (ATF) from Pseudomonas. To obtain ATF most suitable for NMR analysis it was necessary to use HPLC separation instead of the special countercurrent chromatography used earlier. Bioassays for the lytic fraction are done with T. equiperdum isolated from blood of infected rats and purified over a DEAE-cellulose column (Mercado of LPD; Rice, Coleman and Yeh of NIDDK; Ito and Ferrans of NHLBI).

**IMMUNOLOGY OF
STRONGYLOIDIASIS**

Analysis of larval antigens discloses a larval protease. Both somatic and excretion-secretion (E/S) antigens of infective (L3) larvae have been examined by usual techniques, such as silver staining of SDS-PAGE gels, surface iodination, metabolic labelling, etc. In addition, a protease was sought, using gelatin incorporated gels, and found in both somatic and E/S preparations. It is a metallo-protease with activity at 30, 60 and 90 kD, suggesting a trimeric form of enzyme. This protease is undoubtedly required for larval penetration to initiate infection with this parasite. The protease is antigenic and exhibits histamine-releasing activity upon exposure to IgE sensitized basophils, properties which will facilitate further characterization of the material (Brindley, Neva and Pearce of LPD; London and Phillips from SEMA, Inc and McKerrow of Univ. of California).

Possible association of strongyloidiasis with HTLV-1 infection. In the process of our continuing clinical interests in diagnosis and treatment of strongyloides infections, several patients have been encountered with this parasite who also have HTLV-1 infection. Some findings in these patients suggest that HTLV-1 infection may produce selective immune defects that are crucial for control of strongyloidiasis (Neva of LPD, Greenberg and Murphy of NCI).

FROM UNIT ON MICROBIAL PATHOGENESIS

RECEPTORS FOR UPTAKE OF ORGANISMS

Ligands that provide Internalization mechanisms for *T. cruzi*, *Salmonella* and *Toxoplasma*. Since the site of residence of these 3 organisms in phagocytic cells differs (cytosol for *T. cruzi*, phagolysosomes for *S. typhimurium*, and phagosomes for *Toxoplasma*), they provide good models for comparison. Both C1q receptors and fibronectin were found to enhance uptake of *T. cruzi* by macrophages. (Rimoldi, Kipnis and Joiner of LPD, Tenner of Red Cross). Mannose binding protein MBP is an acute phase reactant found in human serum which binds to mannose residues in lipopolysaccharide side chains of bacteria. MBP served as a direct opsonin for ingestion of mannose containing *Salmonella* by human neutrophils, but whether MBP enhances ingestion by macrophages has not yet been tested. There is also some evidence that MBP may activate complement in some fashion. (Joiner and Schweinle of LPD, Ezekowitz of Harvard) Fibronectin binds in a specific fashion to toxoplasma and fibronectin augments attachment and entry of the parasite tachyzoites into macrophages. Surface molecules on the parasite have been identified, and several of them appear to be linked to the tachyzoite cytoskeleton (Fuhrman and Joiner).

Characterization of Intracellular compartments containing intracellular organisms.

Techniques are being developed to label various surface components and phagosomes of neutrophils and macrophages. For example, lactoferrin, a marker for neutrophil secondary granules, was incorporated within phagosomes containing *S. typhi-murium* if coated with IgG, but not if coated with C3. Two different macrophage cell lines, one of which forms phagolysosomes readily and another that is devoid of lysosomal enzymes is being used for these studies (Joiner, Schweinle and Hall of LPD, Rotrosen of LCI).

INTERACTION OF COMPLEMENT WITH MICRO-ORGANISMS

DAF-like molecule produced by *T. cruzi*.

A molecule referred to as decay accelerating factor (DAF) prevents complement activation by both alternative and classical pathways by preventing formation and accelerating decay of C3 convertases. The C3 convertase is an essential part of the complement cascade. A similar DAF molecule produced by trypomastigotes of *T. cruzi*, which helps explain its resistance to killing by serum, was purified and characterized (Joiner, Rimoldi, Kipnis and Sher of LPD and Hammer of LCI).

Complement activation by toxoplasma is non-effective. Although C3 deposition on the surface of *Toxoplasma* occurs in the absence of immune serum, the lytically active C3b is rapidly cleaved to an inactive form. Hence the terminal events of the C cascade are limited and the parasite is not lysed. The limited extent of activation is believed to reflect paucity of glycosylated surface proteins in *Toxoplasma* (Fuhrman, Joiner of LPD and Hammer of LCI).

Re-evaluation of C' activation by gonococci. Previous studies have suggested that gonococci (GC) directly activated the classical C' pathway in the absence of antibody. However, using purified subunits of C1, under conditions in which autoactivation of the C1 subunits is prevented, it was found that GC did not directly activate the classical complement pathway (Schweinle and Joiner).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 0094-29 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Entamoeba histolytica: Nutrition, Differentiation, Virulence, Molecular Probes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: L.S. Diamond Section Head LPD, NIAID

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COOPERATING UNITS (if any)

Department of Biophysics, Weizmann Institute of Science (D. Mirelman) American Type Culture Collection (P.-M. Daggett, T. Nerad), Medical Research Center, VA (A.T. Soldo).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Growth and Differentiation

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.9

PROFESSIONAL:

1.6

OTHER:

1.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither.
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

More than 20 ingredients of PDM-756, a partly defined, serum-free medium for axenic cultivation of E. histolytica are essential for growth of the ameba. In the presence of cysteine and NH_4Cl no other amino acids appear to be required for growth. Radiolabeling studies show amebae grown in PDM-756 are metabolically similar to those grown in standard crude media implying their usefulness for metabolic studies of the parasite.

Southern hybridization and electrophoretic migration of ribosomal DNA through ethidium bromide provided evidence that rRNA genes of E. histolytica are located on extrachromosomal circles. Direct evidence of circular DNA molecules was obtained by electron microscopy. Additionally, two-dimensional agarose gel electrophoretic studies shown the presence of replicating intermediates of the ribosomal DNA circles.

Antigens recognized by humoral responses in patients infected with E. histolytica were identified and characterized by metabolic labeling followed by immunoprecipitation. A major antigen (31-36 kd) was detected with all patients' sera, including sera from cyst passers.

Several monoclonal antibodies produced against cell surface and soluble antigens of E. histolytica recognized antigens from all tested isolates of E. histolytica, but not from other species of Entamoeba, including the non-pathogenic E. histolytica-like Laredo type ameba from humans.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00097-30 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiological and Cytochemical Pathology of Parasitic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	T.I. Mercado	Research Physiologist	LPD, NIAID
Others:	V.J. Ferrans	Chief, Ultrastructure Section	PB, NHLBI
	Y. Ito	Medical Officer	IR, TD, NHLBI
	H.D. Hochstein	Chief, Biol. Testing & Ref. Stds. Branch	CDB, DPOC
	K.C. Rice	Research Chemist	LC, NIDDK
	W.G. Coleman Jr.	Research Microbiologist	LBP, NIDDK
	H.J.C. Yeh	Research Chemist	AC, NIDDK

COOPERATING UNITS (if any)

Waters Life Science Application Laboratory, Fairfax, VA (M.P. Strickler).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

1.0

PROFESSIONAL

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|---|
| ☐ (a) Human subjects | ☐ (b) Human tissues | ☒ (c) Neither |
| ☐ (a1) Minors | | |
| ☐ (a2) Interviews | | |

SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)

Studies on the purification of an anti-trypansomal factor (ATF-II) from the bacterial species, Pseudomonas fluorescens, were continued. It was necessary to modify the purification procedure used previously in order to obtain pure fractions that were suitable for NMR analysis. As before, we used high-speed countercurrent chromatography (CCC) employing the Ito multilayer coiled column but in the high pressure chromatography system (HPLC) we employed both preparative and analytical carbohydrate analysis columns to obtain the NMR fractions. Work is in progress to obtain sufficient amounts of pure compound for these studies. The determination of chemical structure is essential before studies on synthesis are initiated and pharmacological and toxicological properties are examined employing experimental infections with Trypanosoma cruzi.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 0098-32 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Mechanisms of Energy Metabolism in Mammalian and Parasitic Organisms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: E.C. Weinbach

Section Head

LPD, NIAID

Others:

L. Diamond
L. Levenbook
K. Rice
C.H. KimResearch Zoologist
Research Chemist
Research Chemist
Staff FellowLPD, NIAID
LPB, NIDDK
LC, NIDDK
LC, NIDDK

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

4.0

PROFESSIONAL:

2.0

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A study was initiated of the uptake of tricyclic antidepressant drugs by intact trophozoites of Giardia lamblia. Imipramine, a weak drug, was accumulated by the parasite only 20% less than the more potent drug chlorimipramine. This does not account for the 5-fold difference in lethality of the two drugs. Studies are under way to ascertain if the chlorinated derivative, being more lipophilic, traverses the trophozoite membrane and concentrates in subcellular organelles such as lysosomes, to a greater extent than the unsubstituted compound. Mammalian studies of the effects of the halogenated analogs of imipramine (IMP) showed that the 3-bromo derivative was the most effective uncoupler of oxidative phosphorylation in rat liver mitochondria. The order of effectiveness was Br > Cl > F > I > IMP. The halogenated analogs exhibited competitive inhibition of chlorimipramine uptake by G. lamblia trophozoites.

NOTICE OF INTRAMURAL RESEARCH PROJECT

201 AI 00099-13 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biophysical Parasitology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.A. Dvorak Res. Microbiologist LPD, NIAID

Others:	C.P. Mudd	Senior Engineer	ACE, BEIB
	J.J. Bailey	Senior Investigator	LAS, DCRT
	J.C. Engel	Fogarty Fellow	LPD, NIAID
	R. Leapman	Staff Scientist	BEIB
	C. Thompson	Samoff Fellow	LPD, NIAID

COOPERATING UNITS (if any)

Biomedical Engineering and Instrumentation Branch, DRS
Laboratory of Applied Studies, DCRT

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

3.0

PROFESSIONAL

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|---|
| ☐ (a) Human subjects | ☐ (b) Human tissues | ☒ (c) Neither |
| ☐ (a1) Minors | | |
| ☐ (a2) Interviews | | |

SUMMARY OF WORK (Use standard unlined type. Do not exceed the space provided.)

This project is concerned with (a) the genetic diversity of Trypanosoma cruzi and its implications to the epidemiology, course and diagnosis of Chagas' disease, (b) the development of high resolution flow cytometry instrumentation for analyses of cell populations and, (c) the utilization of flow cytometry and low light level video microscopy for the analyses of infectious agents. The marked differences in total DNA/cell between T. cruzi stocks discovered by flow cytometry has been confirmed using conventional diphenylamine analysis. Electron beam x-ray microchemical analyses of the epimastigote stage of T. cruzi clones demonstrate marked interclonal differences in Fe, Zn, S, Mg, K, Ca and P. Spectroscopic analyses reveal that T. cruzi clones with high Fe content also have a proportionately higher cytochrome content than T. cruzi clones with low Fe content. Studies of inter-clonal heterogeneity are being extended to Leishmania spp.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00102-14 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis of Disease Caused by Infection with Intracellular Parasites

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	F.A. Neva	Chief	LPD, NIAID
Others:	D. Sacks	Research Microbiologist	LPD, NIAID
	P. Melby	NRSA Fellow	LPD, NIAID
	Steve James	Senior Investigator	LCI, NIAID

COOPERATING UNITS (if any) Harvard Med. School, Boston, MA (F. von Lichtenberg); Institut de Salubridad y Enfermedades Tropicales, Mexico City (O. Velasco); Univ. of Maryland, Center for Vaccine Development (Stephen Savarino); Rajendra Memorial Institute, Patna, India (Dr. A.B. Sen and Staff); Cooperating units continued on last page

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Cell Biology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During the past year work on this project has mainly involved studies of the immune response to leishmanial infections in patients with different clinical forms of the disease. The survey for cases of diffuse cutaneous leishmaniasis (DCL) in Mexico, which began last year, yielded parasite isolates from 6 DCL cases and 4 from patients with regular cutaneous leishmaniasis (CL). One isolate was L. b. braziliensis, which we believe has not been found before in Mexico; the others were all members of the L. mexicana complex. Since both ulcerative CL and the unusual anergic type of DCL, caused by apparently the same species of organism, occur in the same geographic area, it would appear to be the best area to study host factors responsible for DCL.

Four patients with CL and one new Mexican DCL patient were studied at the Clinical Center. Cryopreserved peripheral blood mononuclear (PBM) cells from regular CL cases were found to give essentially the same proliferative responses a fresh cells. In contrast, cryopreserved PBM cells from a DCL case showed a different pattern, in keeping with our suspicions that the cells responsible for suppression in DCL are more susceptible to damage or destruction by freezing than are most other PBMs. However, more observations on DCL patients are needed to be certain of this conclusion.

Collaborative studies have been initiated with Dr. Ponce in Honduras of an unusual form of cutaneous leishmaniasis due to L. donovani chagasi, the species of parasite normally causing visceral leishmaniasis in Latin America. 16 cases (9 parasitologically confirmed) have occurred in an island community where kala azar has also been present.

Cooperating Units, cont:

Ministry of Health, Tegucigalpa, Honduras (Carlos Ponce); Dept. of Biology, Youngstown University, Ohio (R. Kreutzer); Yale University School of Public Health, Dept. Epidemiology, New Haven, CT (D. McMahon-Pratt).

NOTICE OF INTRAMURAL RESEARCH PROJECT

201 AT 00108-17 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on the Immunobiology of Malaria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	L.H. Miller	Head, Malaria Section	LPD, NIAID
	M.F. Good	Visiting Scientist	LPD, NIAID
	R.W. Gwadz	Senior Scientist	LPD, NIAID
	S. Kumar	Visiting Fellow	LPD, NIAID
Others:	T. McCutchan	Senior Scientist	LPD, NIAID
	W. Weiss	Guest Worker	LPD, NIAID
	F. Dontfraid	Guest Worker	LPD, NIAID

Others continued on last page.

COOPERATING UNITS (if any)

W.L. Maloy, Senior Scientist, LIC, NIAID, J.A. Berzofsky, Senior Scientist, Metabolism Branch, NCI; B. Greenwood, British MRC, The Gambia, West Africa; R. Houghten, M. So, Scripps Clinic, LaJolla, CA; A. Cochrane, NYU Medical Center, NY, NY, R. Wall, USDA, Beltsville, MD.

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

2.9

PROFESSIONAL:

2.5

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Circumsporozoite (CS) proteins have been shown to act as the protective antigens capable of inducing sporozoite-specific immunity in several species of malaria. The gene coding for this antigen in Plasmodium falciparum has been isolated, cloned, and incorporated into E. coli. The recombinant protein induces antibodies in mice which react with sporozoites and blocks liver invasion in vitro.

T cells are critical for malaria immunity, not only because they serve as helper cells for an antibody response, but because they also have an important role as effector cells. The level of the immune response to a given immunogen is determined by Immune Response (*Ir*) genes and tolerance. We have studied the interaction of the malaria parasite with the host immune system and we have found evidence that the parasite has lessened the level of the immune response against it by host mimicry (and resultant immunological tolerance) and by limitation and variation within T sites (i.e. through *Ir* gene control).

Other Investigators cont:

V. de la Cruz
I.A. Quakyi

Staff Fellow
Guest Worker

LPD, NIAID
LPD, NIAID

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00161-11 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunochemistry of Parasitic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T.E. Nash	Medical Officer	LPD, NIAID
Others:	F.A. Neva	Chief	LPD, NIAID
	R.A. Adam	Medical Staff Fellow	LPD, NIAID
	A. Aggarwal	Visiting Fellow	LPD, NIAID
	M. Ballard	Medical Staff Fellow	LPD, NIAID
	A.W. Cheever	Assistant Chief	LPD, NIAID

COOPERATING UNITS (if any)

M.M. Levine, D.A. Herrington, G.A. Losonsky, University of Maryland

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

4.3

PROFESSIONAL:

2.8

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Antigenic variation has previously been documented in vitro using the WB isolate of Giardia lamblia. Similar phenomena occurred in two other isolates indicating antigenic variation occurs generally in Giardia and is not limited to specific isolates. Humans and gerbils were inoculated with defined clones of Giardia and subsequent isolates studied. Both revealed loss of the major surface antigens and replacement with other antigens demonstrating antigenic variation occurs in humans and gerbils. Analysis of humoral responses revealed surface and isolate specific responses.

Ultrasonography was used to study the presence of Symmers' fibrosis in treated and controlled populations compared to untreated villages. A marked decrease of Symmers' fibrosis was found in the treated villages showing treatment of schistosomiasis resulted in a marked decrease in morbidity and mortality.

The need for humoral antibodies in the treatment of cysticercosis with praziquantel was studied in T. taeniaformis infections in mice. Mu treated mice were unable to cure their infection after praziquantel treatment compared to competent mice.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00162-12 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Cytology of Host-Parasite Interactions in Parasitic Protozoa

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	D.M. Dwyer	Supervisory Microbiologist	LPD, NIAID
Others:	P.A. Bates*	Visiting Fellow, FIC	LPD, NIAID
	P.S. Doyle	Visiting Fellow, FIC	LPD, NIAID
	G.O. Gbenle	Internatl. Res. Exchg. Fellow, FIC	LPD, NIAID
	D.J. Mallinson	Visiting Fellow, FIC	LPD, NIAID
	M.O. Odera	Guest Investigator	LPD, NIAID
	K.B. Pastakia	Senior Staff Fellow	LPD, NIAID
	C. McCarthy-Burke	Biologist	LPD, NIAID

COOPERATING UNITS (if any)

*Dept. Zool., Univ. Glasgow; Dept. Immunol. Infect. Dis., Johns Hopkins Univ. (M. Gottlieb); Dept. Biol., Technion-Israel Inst. Tech. (D. Zilberstein); Dept. Biochem., Univ. Victoria (R.W. Olafson); Inst. Med. Parasit., Univ. Bonn (I. Bonsch-Hermes).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology & Cell Biology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

5.7

PROFESSIONAL:

4.7

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

The cell biology and biochemistry of Leishmania is investigated as a model of both intra- and extra-cellular parasitism. As interactions between host and parasite occur at the level of the parasite surface membrane (SM), emphasis is placed on: 1) characterizing its biochemical and physiological functions; and 2) defining its roles in parasite survival and development.

The SM proline-transport protein of L. donovani promastigotes was identified using both direct affinity matrix-binding and anti-idiotypic antibody (anti-ID)-based methods. The anti-ID was generated against an affinity purified anti-polyproline IgG. The anti-ID immunoprecipitated the Leishmania SM protein and it also inhibited proline transport into intact promastigotes by >50%. The SM acid phosphatase was partially purified and characterized. The biosynthetic and secretory mechanisms of the soluble acid phosphatase (SACP) were defined and localized. Antigenic cross-reactivity was demonstrated amongst the SACP's produced by promastigotes of various leishmanial species. Oligonucleotide probes were used to identify and clone the gene(s) for SACP. The SM 3'-nucleotidase was purified to homogeneity and its amino terminus sequenced. The SM 5'-nucleotidase was also isolated, purified and characterized.

The current results provide further functional characterization of parasite SM constituents and these might serve as targets for the design of new chemotherapeutic agents and/or immunodiagnostic/prophylactic reagents.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00197-09 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunoregulation and immune recognition in filariasis and non-filarial diseases.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	T.B. Nutman	Senior Investigator	LPD, NIAID
Others:	E.A. Ottesen	Senior Investigator	LPD, NIAID
	D. Freedman	Medical Staff Fellow	LPD, NIAID
	C.L. King	Medical Staff Fellow	LPD, NIAID
	R. B. Lal	Visiting Scientist	LPD, NIAID

COOPERATING UNITS (if any)

Department of Clinical Immunology, University of Winnipeg, Manitoba, Canada (G. Delespesse);
Department of Onchocerciasis, SNEM, Guatemala City, Guatemala (G. Zea-Flores), Tuberculosis
Research Center, ICMR, Madras, India (P. Narayanan, V. Kumaraswami).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Clinical Parasitology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.2

PROFESSIONAL:

1.0

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to delineate the mechanisms involved in regulating the humoral and cellular responses in patients with filariasis and other disease states. Immunoregulatory studies have examined the phenomenon of antigen-specific anergy in microfilaremic patients by showing this anergy at the level of antibody (B cell) and lymphokine (T cell) responsiveness. Further, similar T cell anergy has been found among patients with active onchocerciasis. The influence of genetic factors, antigen composition, and specific antifilarial chemotherapy on this anergy has begun using molecular biological tools and defined antigens.

In vitro models of parasite-antigen driven antibody production as well as parasite-specific and HTLV-I transformed T cell clones have been developed to understand in more detail those mechanisms regulating antibody production (particularly IgG and IgE) in filarial and non-filarial diseases.

Qualitative analysis of filaria-specific IgE and IgG in loiasis, lymphatic filariasis, and onchocerciasis have indicated patterns of antigen recognition which differ among groups of patients with different clinical manifestations of filariasis. Using these techniques, antigens recognized exclusively by patients with loiasis or onchocerciasis have been identified. Filarial species-specific antigens recognized by human monoclonal antibodies derived from EBV-transformed patient B cells have also been identified and characterized. Further, FPLC fractionation and qualitative analysis of the antigenic components of filarial parasites has identified a two sets of proteins which appears to regulate the immune response at both the cellular and humoral level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00208-08 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Isolation and Characterization of Plasmodial Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	T.F. McCutchan	Microbiologist	LPD, NIAID
Others:	V. de la Cruz	Staff Fellow	LPD, NIAID
	K. Vernick	Technician	LPD, NIAID
	A. Waters	Visiting Associate	LPD, NIAID
	J. Welsh	Biologist	LPD, NIAID

COOPERATING UNITS (if any)

None

LAB BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

5.0

PROFESSIONAL

4.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

This laboratory has been involved in contributing to the development of a malarial vaccine. To this end we have been characterizing surface antigen genes from Plasmodium falciparum. We have found that variation in the circumsporozoite gene of P. falciparum is likely to be biologically selected because of interaction of the parasite surface molecule with host T-cells. We have also studied the ribosomal RNA of different malarial parasites. Information from these studies has important ramifications for the development of malaria diagnostics, the understanding of evolutionary relationships among the different malarial species and the understanding of control of the parasites developmental cycle.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00240-07 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antigenic Analysis of Sexual Stages of Malaria Parasites

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	D. Kaslow	Medical Staff Fellow	LPD, NIAID
	L.H. Miller	Head, Malaria Section	LPD, NIAID
	T.F. McCutchan	Microbiologist	LPD, NIAID

Others:	C. Syin	Guest Worker	LPD, NIAID
	I.A. Quakyi	Guest Worker	LPD, NIAID
	D. Keister	Biologist	LPD, NIAID
	J. Coligan	Branch Chief	BRB, NIAID

COOPERATING UNITS (if any) U. of Colombo, Sri Lanka (K. Mendis); Harvard Sch. of Pub. Hlth, Boston (D. Wirth); Papua New Guinea Inst. Med. Res. (P. Graves); Naval Med. Res. Inst., Bethesda, MD (A. Szafrman); Hazelton Laboratories, VA (J. Rener); U. of Edinburgh, Scotland (R. Carter); Sch. of Pub. Hlth, Hopkins U. (N. Kumar)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

4.6

PROFESSIONAL:

3.2

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects	☒ (b) Human tissues	☐ (c) Neither
☐ (a1) Minors		
☐ (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Three proteins present on the surface of gametes and ookinetes have been identified by monoclonal antibodies as targets of antibodies that block infectivity of gametocytes to mosquitoes. We cloned one of these proteins by immunoaffinity purifying the protein, microsequencing tryptic peptides of the protein, and then screening genomic libraries with oligonucleotides. The methods for purifying the other target proteins for microsequencing are currently being developed. We have continued the search for novel target antigens of antibodies that block transmission of sexual stage malaria parasites, and have identified a parasite molecule that may be involved in release of gametocytes from red blood cells.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00241-07 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Identification of Receptors for Merozoite Invasion of Erythrocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)

PI:	L.H. Miller	Section Head	LPD, NIAID
Others:	J. Adams	Biologist	LPD, NIAID
	D. Hudson	Biologist	LPD, NIAID
	T. Welles	Sr. Staff Fellow	LPD, NIAID

COOPERATING UNITS (if any)

WRAIR, Washington, DC (F. Klotz and J.D. Haynes); Hazelton Laboratories, Vienna, VA (J. Renner); Case Western Reserve Univ., Cleveland, OH (M. Aikawa); Univ. of Kentucky Sch. of Med. (T. Hadley); University of Dublin (J. Dalton)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

2.4

PROFESSIONAL

1.4

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The merozoite interacts in a receptor specific manner with the erythrocyte surface and is the stage against which immunity may work to block invasion. Thus, merozoite surface components are of interest for their role in erythrocyte recognition and as antigens for induction of protective immunity. We are identifying P. knowlesi receptors for attachment to monkey and human erythrocytes and P. falciparum proteins that are involved in the junction between merozoites and erythrocytes. We are studying one antigen on the merozoite surface that undergoes antigenic variation to understand the molecular basis for this variation. We are attempting to adapt P. falciparum to subprimates as an animal model for vaccine trials.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological and biochemical studies of antigens on malaria-infected red cells.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: R. Howard Visiting Scientist LPD, NIAID

Others:	L.H. Miller	Section Head	LPD, NIAID
	L.J. Panton	Visiting Fellow	LPD, NIAID
	A.S. De Groot	Clinical Associate	LPD, NIAID
	W. Daniel	Research Associate	LPD, NIAID

COOPERATING UNITS (if any)

Case Western Reserve University (M. Aikawa, C. Atkinson); Christian-Albrecht's University, West Germany (R. Schauer, G. Reuter); University of California San Francisco (J. Leech, P. Rosenthal); DNAX Research Institute, Palo Alto (T. Yokota, K. Moore).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.6

PROFESSIONAL:

0.4

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|---|
| ☐ (a) Human subjects | ☐ (b) Human tissues | ☒ (c) Neither |
| ☐ (a1) Minors | | |
| ☐ (a2) Interviews | | |

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

We are studying the structure and function of malarial proteins inserted into and through the membrane of erythrocytes infected with mature asexual parasites and molecules on the endothelial cells involved in cytoadherence.

Molecular biological approaches are being developed for attempted cloning of the P. knowlesi variant antigen on infected erythrocytes and the P. falciparum protein PfEMP1 that our earlier studies implicated in the cytoadherence property of these cells.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00244-07 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on the Developmental Adaptation of *Trypanosoma cruzi* to the Vertebrate Immune System.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	A. Sher	Section Head	LPD, NIAID
Others:	R. Da Silva	Fogarty Fellow	LPD, NIAID
	S. Heath	Fogarty Fellow	LPD, NIAID
	K. Joiner	Senior Investigator	LPD, NIAID
	M. Grimaldi	Fogarty Fellow	LPD, NIAID
	D. Sacks	Senior Investigator	LPD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

2.5

PROFESSIONAL

2.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In this project we have been studying developmental adaptations of *T. cruzi* to the vertebrate host and, in particular, the molecular basis of adaptive changes occurring during the differentiation of epimastigotes (vector stage) to metacyclic trypomastigotes (infective stage).

A. Studies on developmental control of intracellular survival in macrophages

Epimastigotes were shown to enter mouse macrophages by CR3 receptors while the entry and survival of metacyclic trypomastigotes was shown to be enhanced by complement but also to depend on fibronectin receptors.

B. Analysis of stage-specific gene expression in metacyclic forms

Metacyclic stage specific cDNA's were cloned from *T. cruzi* and *L. major*. Sequence analysis indicated that the principal *T. cruzi* clone has homology with a c-fos proto-oncogene while the *L. major* clone has homology with β -chain of RNA polymerase.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00246-06 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Studies of the Genome and Surface of Schistosoma mansoni

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	A. Sher	Section Head	LPD, NIAID
Others:	E. J. Pearce	Visiting Associate	LPD, NIAID
	M. Knight	Special Volunteer	LPD, NIAID
	L. Hall	Medical Staff Fellow	LCI, NIAID
	A. Cheever	Assistant Chief	LPD, NIAID
	J. Coligan	Chief	BRB, NIAID

COOPERATING UNITS (if any)

Biomedical Research Institute, Rockville, MD (F. Lewis).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.1

PROFESSIONAL:

1.6

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of this project is to characterize schistosome surface molecules relevant to immunity, to analyze the genome of the parasite in relation to specific biological properties of the organism and to clone important schistosome immunogens.

A. Molecular biology of paramyosin. Paramyosin was shown to be encoded by an unusually large transcript. cDNA's comprising unsequenced portions of the molecule were isolated along with peptides in these same regions.

B. Demonstration of lipid anchors on schistosomulum membrane proteins. Four major schistosomulum surface antigens of Mr 70k, 32k-38k and 18k were shown to be anchored in the tegument by phosphatidylinositol linkages and released in the form of membrane vesicles.

C. Detection of host DAF on the schistosome membrane. Using specific antibodies, host decay accelerating factor (DAF) was identified in the membrane of schistosomes recovered from monkeys and guinea pigs.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00248-07 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetics & Physiology of Vector Capacity in Anopheline Mosquitoes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R.W. Gwadz	Senior Scientist	LPD, NIAID
	L.H. Miller	Section Head	LPD, NIAID
	R.K. Sakai	Expert	LPD, NIAID
Others:	P.A.J. Romans	Visiting Fellow	LPD, NIAID
	K.D. Vernick	Bio. Lab. Tech.	LPD, NIAID
	D. Kaslow	Med. Staff Fellow	LPD, NIAID
	J. Y. Lee	Visiting Fellow	LPD, NIAID

Others continued on last page.

COOPERATING UNITS (if any)

Dept. of Entomology, U. of Maryland, College Park, MD (Dr. M. Ma); Institute of Parasitology, U. of Rome (Dr. M. Coluzzi); Institute of Pathology, Case Western Reserve School of Medicine (Dr. M. Aikawa); Ecole Nationale de Medecine, Bamako, Mali (Dr. Y.T. Toure); WRAIR (K.L. Sim)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

7.9

PROFESSIONAL:

6.1

OTHER:

1.8

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The molecular biology of anopheline mosquitoes is being studied in relation to the capacity of these vectors to transmit malaria. Systems for cloning and transposing genes into mosquito germ lines are being developed. Factors which render mosquitoes refractory to malarial infection, with special emphasis on mechanisms which encapsulate developing parasites, are being defined. Linkage of refractory genes to genes regulating blood-meal associated physiological events, e.g., vitellogenin synthesis, will be used to enhance refractory responses.

Additional parasitocidal mechanisms from within and outside of mosquitoes are being sought with special emphasis on the evaluation of magainins and cecropins, their physiological effects on in vivo parasite development and the molecular biology of the genes coding for their production.

The ability to identify, clone, and transpose genes which regulate refractory mechanisms should permit the development of mosquito lines incapable of transmitting malaria for use in malaria control schemes.

Other Investigators cont.:

Others:	H.G. Coon	Senior Investigator	LG, NCI
	P. Kantoff	Senior Investigator	LMH, NHLBI
	K. Mizuuchi	Senior Investigator	LMB, NIDDK
	K.-P. Sieber	Staff Fellow	LPD, NIAID
	M. Huber	Visiting Fellow	LPD, NIAID
	C. Graziosi	Guest Worker	LPD, NIAID

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00251-07 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunologic Studies on Schistosomiasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Sher	Section Head	LPD, NIAID
Others:	E. J. Pearce	Guest Worker	LPD, NIAID
	P. Brindley	Fogarty Fellow	LPD, NIAID

COOPERATING UNITS (if any)

George Washington University (S.L. James); Johns Hopkins University (M. Strand); Fiocruz, Belo Horizonte, Brazil (R. Correa-Oliveira).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.8

PROFESSIONAL:

2.0

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
- ☒ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of this project is to study mechanisms of immunity and immune evasion in schistosomiasis with the ultimate goal of developing an experimental vaccine employing defined antigens.

A. Vaccination trials with purified paramyosin and GST. Paramyosin and glutathione-S transferase (GST) were both shown to induce protective immunity in mice using either BCG or saponin as adjuvants, while combining the two antigens had no additive effect.

B. Recognition of paramyosin by endemic populations. Patients in a Brazilian field population were screened for antibodies against paramyosin. Asymptomatic, non-infected individuals were found to develop higher responses than infected patients while hepato-splenic patients developed lower responses on average than either group.

C. Characterization of non-paramyosin T cell protective immunogens. Non-paramyosin molecules which could serve as immunogens for protective immunity were characterized using T cell immunoblotting and chromatographic techniques.

D. Role of antibodies against Sm-200 in praziquantel therapy. In passive transfer studies a monoclonal antibody against a 200 kD tubercle glycoprotein was shown to restore the ability of u-suppressed mice to eliminate adult worms when treated with praziquantel.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00253-07 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT: 80 characters or less. Title must fit on one line between the borders.

Studies of the Immunological Responses to Filarial Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: E.A. Ottesen Senior Investigator LPD, NIAID

Others:	T.B. Nutman	Medical Officer	LPD, NIAID
	D. Freedman	Medical Staff Fellow	LPD, NIAID
	R. Hussain	Visiting Fellow	LPD, NIAID
	R. Davey	Medical Staff Fellow	LCI, NIAID
	R. Lal	Visiting Fellow	LPD, NIAID

COOPERATING UNITS (if any): Harvard Med Sch (S Ackerman); Walter Reed Army Inst Res (M Grogli); PB, NHLBI, NIH (RG Crystal & W Rom); CB, NEI, NIH (R Nussenblatt & C Chan); Indian Cncl Med Res Tuberculosis Res Centre, Madras, India; (S Tripathy, P Narayanan, V Kumaraswami, R Paranjape, V Vijayan); Madras Med Coll (S Jamal); Dept Hlth, Guatemala (G Zea-Flores).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host Parasite Relations

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.6

PROFESSIONAL:

2.0

OTHER:

1.6

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to define the humoral and cellular immune responses that relate to immunopathology, protective immunity and immunodiagnosis of patients with lymphatic filariasis and onchocerciasis.

Antibodies of the IgG subclasses appear differentially associated with the clinical manifestations of bancroftian filariasis; patients with elephantiasis have prominent IgG₃ antifilarial antibodies and minimal IgG₄ responses while the opposite is true for those with asymptomatic infection. These IgG₄ antibodies appear to be the "blocking antibodies" responsible for inhibiting IgE-mediated immediate hypersensitivity responses in vitro and in vivo, and they have also proven to be useful diagnostically.

The eosinophil granule proteins MBP and ECP, important in the pathogenesis of helminth infections, can now be defined in tissues by immunofluorescence (MBP) and in fluids by ELISA with monoclonal antibodies.

Populations with bancroftian filariasis or onchocerciasis have been examined to define immunologic parameters that distinguish "naturally" immune from infected individuals. A 43kD protein from infective larvae appears as a potential protective immunogen for bancroftian filariasis, and specific probes are being generated. Enhanced T-cell reactivity to parasite antigen was the marker most clearly distinguishing putatively immune individuals from those with overt onchocerciasis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00255-07 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the Immunologic Responses to Non-Filarial Helminth Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	E.A. Ottesen	Senior Investigator	LPD, NIAID
Others:	T.B. Nutman	Medical Officer	LPD, NIAID
	D.O. Freedman	Medical Staff Fellow	LPD, NIAID
	A.W. Cheever	Assistant Chief	LPD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host Parasite Relations

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To account for the neo-arteriolization of the peri-portal fibrotic tissue around the eggs of Schistosoma mansoni in the livers of infected humans, parasite products were evaluated for endothelial cell growth factor activity. Very potent endothelial cell activation and proliferation were induced by soluble antigens from eggs (SEA) but not adult schistosomes, all in the absence of any immune cells or factors. SEA from S. japonicum also stimulated endothelial proliferation but to a much lesser degree than did that of S. mansoni; S. mekongi and S. hematobium SEA were ineffective in stimulating these cells to proliferate. All the angiogenic factors thus far elucidated have been derived from mammalian sources. That a parasitic invertebrate could be capable of producing a unique angiogenic substance or be able to mimic one normally produced by its host has important biological implications.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00256-07 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Differentiation of Leishmania Promastigotes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	D. Sacks	Senior Investigator	LPD, NIAID
Other:	R. da Silva	Fogarty Fellow	LPD, NIAID
	S. Puentes	Clinical Staff Fellow	LCI, NIAID
	L. Hall	Clinical Staff Fellow	LCI, NIAID
	K. Joiner	Senior Investigator	LCI, NIAID

COOPERATING UNITS (if any)

U. Kentucky (S. Turco).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

1.8

PROFESSIONAL

1.8

OTHER:

0.0

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

The differentiation of L. major promastigotes from a non-infective to an infective or metacyclic stage is accompanied by changes in surface carbohydrates which can be detected by the lectin peanut agglutinin (PNA). On non-infective, logarithmic phase promastigotes, PNA binds to the surface lipophosphoglycan (LPG). During metacyclogenesis, the LPG is developmentally modified such that it no longer binds PNA, it expresses a novel carbohydrate epitope, and it is substantially increased in size. A major difference between the two forms can be localized to the composition of the tetrasaccharide subunits of the phosphorylated carbohydrates.

The manner in which this structural change promotes complement resistance and intracellular survival is being investigated. The major acceptor molecule for C3b deposition on metacyclics is the LPG, which remain resistant to lysis because the membrane attack complex, while formed, is released from the surface. Efficient deposition of C3b in the absence of lysis promotes uptake of opsonized promastigotes by macrophage CR1 receptors.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00257-07 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunology of Strongyloidiasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	F.A. Neva	Chief	LPD, NIAID
Others:	P.J. Brindley	Special Volunteer	LPD, NIAID
	E. Pearce	Visiting Associate	LPD, NIAID
	W.T. London	Chief, Exptl. Path. Sect.	IRP, NICDS
	S. Greenberg	Medical Staff Fellow	MB, NCI
	E.L. Murphy	Medical Staff Fellow	EPB, NCI
	T. Nash	Senior Investigator	LPD, NIAID
	E. Ottesen	Senior Investigator	LPD, NIAID

COOPERATING UNITS (if any)

Univ. of California at San Francisco, Dept. Pathology (J. McKerraw); SEMA, Inc., Rockville (G. Phillips); Merck, Sharpe, Dohme, Rahway, NJ (K. Brown).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Cell Biology and Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.3

PROFESSIONAL:

0.8

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project involves clinical and immunologic studies of humans infected with the intestinal nematode, Strongyloides stercoralis, and parallel studies in an experimental host, the patas monkey. Research on this parasite is justified because human infections occur in substantial numbers even in the U.S., it often goes undiagnosed and can produce fatal outcome in immunosuppressed people, and it is a parasite with unusual biologic properties.

A good portion of the somatic and E/S antigens obtained from infected patas monkeys in the past year is being stockpiled for a new batch of skin test antigen. Antigenic analysis of infective larvae (L3) has focused heavily upon identification and characterization of a protease present in both somatic and E/S antigens. It is a metallo-protease, with functional activity demonstrable at about 30, 60, and 90 kD, suggesting a trimeric form. The protease is also allergenic - i.e., it causes histamine release from IgE sensitized basophils in-vitro. Monoclonal antibodies raised to somatic larval antigen have been relatively weak in identifying antigens, and IgE Western blots are faint so that these reagents and approaches have not been as useful as expected. The possibility that HTLV-1 infection may selectively impair immune responses important for control of strongyloidiasis in some patients is being investigated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00277-05 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of Serum Resistance in Bacteria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	K. Joiner	Senior Investigator	LPD, NIAID
Others:	J.E. Schweinle	IPA	LPD, NIAID

COOPERATING UNITS (if any)

Andrea Tenner, American Red Cross

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Unit of Microbial Pathogenesis

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

0.2

PROFESSIONAL:

0.2

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|---|--------------------------------------|
| ☐ (a) Human subjects | ☒ (b) Human tissues | ☐ (c) Neither |
| ☐ (a1) Minors | | |
| ☐ (a2) Interviews | | |

SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)

Studies were completed on the opsonic and bactericidal activity of a monoclonal antibody directed against the outer membrane proteolipid, H.8, of Neisseria gonorrhoeae. In addition to its anti-gonococcal and opsonic activity described in earlier reports, it was found to override the regulatory function of the serum glycoprotein C1 inhibitor and to allow activation of C1 with sequential activation of the classical complement pathway. Despite early suggestions that Neisseria gonorrhoeae directly activated the classical complement pathway, meticulous studies prohibiting spontaneous activation of C1 by autocatalytic mechanisms proved otherwise. Qualitative measurement of state of activation of C1s proved that Neisseria gonorrhoeae does not directly activate the classical complement pathway in the absence of antibody whether or not C1 inhibitor is present. When antibodies are included in the assay, activation always occurs.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00347-06 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Schistosomal Hepatic Fibrosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: A. W. Cheever Assistant Chief LPD, NIAID

Others:	S. Deb	Bio. Lab. Tech.	LPD, NIAID
	R. H. Duvall	Bio. Lab. Tech. (Micro.)	LPD, NIAID
	J. Malley	Mathematical Statistician	LSM, DCRT
	A. Sher	Head, Immunology Section	LPD, NIAID
	R. Minker		LSM, DCRT
	P. Scott	Senior Staff Fellow	LPD, NIAID

COOPERATING UNITS (if any)

Laboratoire de Pathologie Cellulaire du Foie, Institut Pasteur, Lyon (Jean-Alexis Grimaud).
Division of Infectious Diseases, George Washington University (Carmelita Tuazon, Ann Labriola).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

0.50

OTHER:

0.50

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Hepatic fibrosis and the granulomatous response to eggs of schistosome species pathogenic for man are studied in mice in relation to parasitologic parameters of infection. Hepatic fibrosis increases in the first weeks after treatment of S. japonicum infection in mice and then remains stable for at least 1 year following therapy. During this period histologic reactions to eggs and dead worms involute and the areas of fibrosis become smaller but denser. Mice vaccinated with paramyosin and BCG showed no significant difference in granuloma size or hepatic fibrosis compared to nonvaccinated mice.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00350-06 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

DNA Analysis of Parasites

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: T.E. Nash Medical Officer LPD, NIAID

Others:	A. Aggarwal	Visiting Fellow	LPD, NIAID
	R. Adam	Medical Staff Fellow	LPD, NIAID
	T. McCutchan	Senior Scientist	LPD, NIAID
	T. Wellem	Staff Fellow	LPD, NIAID
	V. de la Cruz	Staff Fellow	LPD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.2

PROFESSIONAL:

1.7

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|---|
| ☐ (a) Human subjects | ☐ (b) Human tissues | ☒ (c) Neither |
| ☐ (a1) Minors | | |
| ☐ (a2) Interviews | | |

SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)

Giardia surface antigens undergo antigenic variation. To determine the relatedness of one variant to another, cDNA libraries of two related variants were successfully produced and screened with surface specific Mab or previously defined DNA probes. Analysis and sequencing are in progress. Pulse field gradient analysis of Giardia chromosomes continue. One minor band was related to one of the major chromosomes. Markers were derived product is recognized by all infected animals and humans. SEquencing of this gene revealed no relationship to other proteins. This antigen may be valuable for detecting Giardia specific antibody responses. Attempts to transform Giardia continue. Constructs were made using a Giardia heat shock promoter and the lacZ gene and used to transform Giardia. Giardia were transformed transiently.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00351-06 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Parasite and Host Factors Controlling the Pathogenesis of Leishmaniasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P.A. Scott	Expert	LPD, NIAID
Others:	A. Sher	Section Head	LPD, NIAID
	F.A. Neva	Chief	LPD, NIAID
	P. Natovitz	Biologist	LPD, NIAID
	D. Dwyer	Supvr. Microbiologist	LPD, NIAID
	E. Pearce	Guest Researcher	LPD, NIAID
	S. Heath	Guest Researcher	LPD, NIAID
	M. Knight	Guest Researcher	LPD, NIAID

COOPERATING UNITS (if any)

George Washington University, Washington, DC (S. James); DNAX Research Institute, Palo Alto, CA (R. Coffman).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.5

PROFESSIONAL:

1.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

A vaccine against *Leishmania major* in BALB/c mice using a soluble fraction of the parasite was developed. Further fractionation of this soluble leishmanial antigen (SLA) yielded both protective (fraction 9) and non-protective (fraction 1) fractions that were capable of stimulating T cells. To evaluate the nature of the T cells recognizing fractions 1 and 9, as well as to define protective leishmanial antigens, T cell lines were established against both antigen preparations. A line established against fraction 9 (Line 9) was found to adoptively transfer protection, while a line recognizing fraction 1 (Line 1) not only failed to protect mice, but exacerbated the infection. We found that in addition to differing in their antigen specificity, these T cell lines could be distinguished by their lymphokine profile. Upon stimulation Line 9 produced IL-2 and IFN-gamma, while Line 1 produced IL-4 and IL-5. This pattern corresponds to TH1 (Line 9) and TH2 (Line 1) helper T cell subsets. In order to determine why Line 1 exacerbated leishmanial infection, we have tested the ability of supernatants derived from these cells to influence parasite growth and killing within macrophages, and have found that Line 1 supernatants contain a factor(s) that downregulates the ability of macrophages to be activated by IFN-gamma. Studies are in progress to define the T cell products responsible for this effect. To define the protective antigens within fraction 9, we established a T cell clone from Line 9 and by using T cell immunoblotting techniques demonstrated that it recognized a protein of approximate m.w. 10,000. We have further demonstrated that this clone adoptively transfers protection equivalent to that obtained with Line 9. Two strategies are currently being employed to obtain a cDNA clone of this molecule. The first is production of monoclonal antibodies and the second is isolation of sufficient material for N-terminal sequencing, and subsequent development of an oligonucleotide for screening.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00398-03 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (30 characters or less. Title must fit on one line between the borders.)

Studies on the Interaction Between Complement and Parasites

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)

PI:	K. Joiner	Senior Investigator	LPD, NIAID
Others:	S. Puentes	Medical Staff Fellow	LPD, NIAID
	M.T. Rimoldi	Fogarty Fellow	LPD, NIAID
	S. Fuhman	Medical Staff Fellow	LPD, NIAID
	C. Hammer	Senior Staff Fellow	LPD, NIAID
	D. Sacks	Senior Staff Fellow	LPD, NIAID

COOPERATING UNITS (if any)

none

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Unit of Microbial Pathogenesis

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MANY YEARS:

1.3

PROFESSIONAL:

1.3

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)

Experiments were completed examining the interaction of complement with Trypanosoma cruzi, Leishmania major, and Toxoplasma gondii.

Final purification and characterization of the C3 convertase inhibitor produced by trypomastigotes of T. cruzi was completed. The molecule is an 87-93kd glycoprotein, P.I. 5.6-5.8, expressed in infective trypomastigote but not non-infectious epimastigote forms. Functionally, the trypomastigote molecule is identical to human decay accelerating factor: it prevents formation and accelerates decay of the classical and alternative pathway C3 convertases. There is a correlation between the lytic activity of Chagasic serum and the capacity of antibodies in the serum to recognize the C3 convertase inhibitor by immunoprecipitation and to block the functional activity of the inhibitor.

The mechanism of resistance to serum killing for metacyclic promastigotes of Leishmania major was further clarified. Both serum sensitive log phase promastigotes and serum-resistant metacyclic promastigotes activate complement efficiently, and a C5b-9 complex is formed on the parasite membrane. With metacyclic promastigotes, the C5b-9 complex is shed and is not lytic because it fails to insert into hydrophobic domains of the Leishmania membrane.

Tachyzoites of Toxoplasma gondii are resistant to serum killing because limited C3 and C5b-9 are deposited during incubation in serum. Bound C3b is rapidly cleaved to the lytically inactive iC3b form. Complement deposition is increased and the site of deposition is altered by lytic monoclonal antibodies for tachyzoites.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00439-04 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical and Therapeutic Studies in Human Filariasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	E.A. Ottesen	Senior Investigator	LPD, NIAID
Others:	T.B. Nutman	Medical Officer	LPD, NIAID
	D.O. Freedman	Medical Staff Fellow	LPD, NIAID
	T.E. Nash	Senior Investigator	LPD, NIAID
	R. Davey	Medical Staff Fellow	LPD, NIAID

COOPERATING UNITS (if any)

PB, NHLBI, NIH (R.G. Crystal); Tuberculosis Research Centre, Madras, India; (R. Prabhakar and V. Kumaraswami); Madras Medical College, Madras, India (V. Vijayasekaran); Peace Corps Medical Office (M. Mulligan).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host Parasite Relations

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.6

PROFESSIONAL:

0.5

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Ivermectin has been shown in an open, in-hospital trial in South India to be effective in clearing microfilaremia in patients with bancroftian filariasis at dosages as low as 25-mcg/kg given once orally. A second, blinded, placebo-controlled trial of ivermectin and the currently used drug, diethylcarbamazine (DEC), has indicated that the efficacy of ivermectin and DEC were equivalent for 3 months, but by 6 months the ~30% microfilarial recurrence rate of the ivermectin-treated patients was significantly greater than the ~15% rate of the DEC group. Side effects were qualitatively and quantitatively identical for both groups. Thus, though ivermectin appeared somewhat less effective than DEC at 6 months, its single-oral-dose mode of administration with toxicity no greater than that of DEC means that this drug should engender greater patient compliance and, therefore, be much more effective in mass-treatment, filariasis control programs than DEC.

Observations on 200 Peace Corps volunteers going to Loa loa endemic areas of Africa have concluded after three years of a placebo-controlled chemoprophylaxis trial using weekly doses of DEC. In Gabon (where exposure to the parasite was heaviest) 30% of individuals in the placebo group developed overt clinical disease compared to none in the DEC-treated group ($p < .02$) and 50% became seropositive in the placebo group vs. 13% in the DEC group ($p < .02$). No significant side effects were seen. These findings indicate that DEC given orally once-weekly can be an effective, acceptable chemoprophylactic for preventing loiasis.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00484-03 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetics of Drug Resistance and Red Cell Invasion in Malaria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T.E. Wellems	Senior Staff Fellow	LPD, NIAID
Others:	L.J. Panton	Fogarty Fellow	LPD, NIAID
	D. Peterson	Research Fellow	LPD, NIAID
	S. Dolan	Medical Staff Fellow	LPD, NIAID
	R. Gwadz	Senior Investigator	LPD, NIAID
	P. Sinnis	Visiting Fellow	LPD, NIAID

COOPERATING UNITS (if any)

Virgilio E. do Rosario Biomedical Research Institute

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

4.0

PROFESSIONAL

3.5

OTHER:

0.5

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our investigations concern the resistance of *Plasmodium falciparum* to several common antimalarial drugs: the dihydrofolate reductase inhibitor pyrimethamine, and the quinoline ring derivatives chloroquine, quinine, quinidine and mefloquine. We are using crosses of cloned parasite lines to identify the genes that affect susceptibility to these drugs. Point mutations in the gene encoding dihydrofolate reductase-thymidylate synthase have been found to confer pyrimethamine resistance in malaria. Chloroquine resistance has been found to be governed by a single gene in linkage studies. RFLP analysis is being used to pinpoint the location of this gene.

A similar approach is being used to identify genes affecting erythrocyte-stage proliferation. In a cross between two clones the faster proliferation rate of one parent predominated in progeny recovered after cross fertilization and infection of chimpanzees. Linkage group analysis suggests an association between a genetic locus near a telomere of chromosome 13 and the rapid proliferation rate. Investigations are now planned to map the genes involved and examine for relationship to sialic acid and erythrocyte-binding proteins thought to modulate erythrocyte invasion by *P. falciparum*.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00487-02 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on the Quantitative Parasitology of Schistosome Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. W. Cheever Assistant Chief LPD, NIAID

Others: S. Deb Bio. Lab. Tech. LPD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

0.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Eggs in the tissues of S. japonicum infected mice persisted in unchanged numbers in the tissues for at least 1 year after chemotherapeutic cure of infections of 8 weeks duration. Worm burdens decreased with time in S. mansoni infected mice between the 8th and 20th week of infection. No dead worms were found in the liver and the decrease in worm burden was caused by death of more heavily infected mice and shunting of worms from the portal system to the pulmonary arterial circulation.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00494-02 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of T Cell Responses in Human Leishmaniasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	D. Sacks	Senior Investigator	LPD, NIAID
Others:	P. Melby	Staff Fellow	LPD, NIAID
	F. Neva	Chief	LPD, NIAID
	A.B. Sen	Director	RMRI, Patna, India

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Cell Biology and Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

14

PROFESSIONAL:

14

OTHER:

00

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
☐ (b) Human tissues
☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Control and resolution of leishmanial infection depends primarily on T cell mediated immune mechanisms. The nature of the leishmanial antigens involved in eliciting T cell immunity has been studied by the recently developed method of cellular immunoblotting. In the past year, we have modified this method to include the analysis of human T cell responses to nitrocellulose-bound antigens highly resolved by two-dimensional electrophoresis. The proliferative responses of cells from patients with mucosal, and cutaneous leishmaniasis were remarkably heterogeneous and occurred to as many as 50-70 distinct antigens, many of which were associated with γ -interferon production. The method has also been used to identify specific 2-D resolved antigens which stimulate T cell clones derived from patients with acquired resistance to leishmaniasis. We are now attempting to obtain sequences off of these immobilized blotted proteins in order to construct oligonucleotide probes so that genes containing these T cell epitopes might be cloned. Recently, intralesional T lymphocyte lines and clones have been produced and their antigen specificity will be profiled and compared with that displayed by peripheral cells from the same patient.

Finally, studies are soon to be initiated using cDNA probes for cytokine mRNA expressed by activated lymphocytes from patients with mucosal and cutaneous leishmaniasis were remarkably heterogeneous and occurred to as many as 50-70 distinct antigens, many of which were associated with γ -interferon production. The method has also been used to identify specific 2-D resolved antigens which stimulate T cell clones derived from patients with acquired resistance to leishmaniasis. We are now attempting to obtain sequences off of these immobilized blotted proteins in order to construct oligonucleotide probes so that genes containing these T cell epitopes might be clones. Recently, intralesional T lymphocyte lines and clones have been produced and their antigen specificity will be profiled and compared with that displayed by peripheral cells from the same patient.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 00512-01 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Definition of Filarial Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: T.B. Nutman Senior Investigator LPD, NIAID

Others: N. Raghavan Visiting Scientist LPD, NIAID
K.F. Colina Howard Hughes Research Scholar LPD, NIAID
E.A. Ottesen Senior Investigator LPD, NIAID

COOPERATING UNITS (if any)

New England Biolabs, Beverly, Mass (F. Perler, P. Arasu, L. McReynolds, C. Maina), Albert Einstein Coll. Med., Bronx, N.Y. (T.V. Rajan)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Clinical Parasitology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

2.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In the study of the human filariases, progress has been hampered by: 1) the lack of defined parasite antigens; 2) the broad immunological cross-reactivity seen among the eight filarial species of humans; and 3) the dearth of abundant parasite material. The objectives of this project are to define and generate filarial proteins that are important in inducing parasite-specific immune responses in the human host and to understand, at a molecular level, the differences among related filarial species.

cDNA and genomic libraries have been either constructed (Brugia malayi and Wuchereria bancrofti) or made available (Onchocerca volvulus) so that screening with defined patient sera or patient T cells could be performed. Recombinant antigens and probes have been identified that: a) induce T cell responses in an antigen-specific manner; b) may be in part protective in onchocerciasis; c) can distinguish among related filarial species by restriction fragment length polymorphisms; d) encode for an immunodominant molecule in W. bancrofti; and e) identify a tandemly repeated segment of the W. bancrofti genome.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00517-01 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the brackets)

Ligand-Receptor Interactions in Parasite Attachment to Cell

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Keith Joiner Senior Investigator LPD, NIAID

Others:	Steve Fuhman	Medical Staff Fellow	LPD, NIAID
	M.T. Rimoldi	Fogarty Fellow	LPD, NIAID
	G. Furtado	Guest Researcher	LPD, NIAID
	J. Schweinle	IPA	LPD, NIAID
	T. Kipnis	Guest Worker	LPD, NIAID

Others continued on next page.

COOPERATING UNITS (if any)

Andrea Tenner, American Red Cross; Alex Kurosky, Univ. of Texas, Galveston Branch; Ken Ingham, American Red Cross, Rockville, MD; Alan Ezekowitz, Children's Hospital, Harvard Univ.

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Unit on Microbial Pathogenesis

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

3.1

PROFESSIONAL

3.0

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The major ligand-receptor systems involved in uptake of the intracellular pathogens *Trypanosoma cruzi*, *Salmonella typhimurium*, and *Toxoplasma gondii* by phagocytic cells were examined. For *T. cruzi*, incubation of infective trypomastigotes in normal human serum does not lead to parasite lysis but markedly enhances entry into macrophages and monocytes. This enhancement is due to deposition of complement component C1q on the parasite, and the additional interaction of C1q with fibronectin bound to the parasite. C3 fragments play no role in enhancing internalization. Trypomastigotes are thus targeted to cells bearing large numbers of fibronectin and C1q receptors, of which fibroblasts and epithelial cells are the prototype.

Salmonella typhimurium, and other *Salmonella* species attach to and enter phagocytic cells via receptors for C3 fragments. Mannose binding protein, an acute phase reactant in serum which binds to mannose on selected microbial surfaces, is a direct opsonin for *Salmonella* containing mannose in the lipopolysaccharide. Mannose binding protein also enhances complement activation on the surface of mannose bearing strains.

Toxoplasma gondii attachment and entry into macrophages is augmented by fibronectin. Furthermore, fibronectin binds in specific fashion to the parasite. A surface iodinated 16-18kd molecule on the tachyzoite, shown to be anchored to the parasite cytoskeleton, has been purified to homogeneity. This role of this molecule in binding fibronectin and in cell entry is under investigation.

Other Investigators cont.

B.F. Hall
G.B. Ogden
A.B. Tartanian

Medical Staff Fellow
Senior Staff Fellow
Technician

LPD, NIAID
LPD, NIAID
LPD, NIAID

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00535-01 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Intracellular Compartments Containing Micro-organisms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Keith Joiner	Senior Investigator	LPD, NIAID
Others:	B.F. Hall	Medical Staff Fellow	LPD, NIAID
	J.E. Schweinle	IPA	LPD, NIAID
	D. Rotrosen	Medical Staff Fellow	LCI, NIAID
	A.B. Tartanian	Technician	LPD, NIAID

COOPERATING UNITS (if any)

none

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Unit on Microbial Pathogenesis

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

Our laboratory is interested in the consequences of particular ligand-receptor interactions which mediate cell entry on the ultimate intracellular compartmentalization and fate of obligate intracellular microorganisms.

Methods were developed to purify and characterize phagosomes from human neutrophils ingesting Salmonella typhimurium. Taking advantage of the fact that neutrophils will endogenously iodinate particles which are phagocytosed in the presence of ^{125}I Na, we characterized iodinated constituents in purified phagosomes containing Salmonella typhimurium. Bacterial constituents, humoral ligands, and cell surface receptors for C3 fragments and for IgG were iodinated and incorporated into the phagosomes regardless of whether the ligand on Salmonella was C3 or IgG. Of particular interest, lactoferrin, a marker for neutrophil secondary granules, was iodinated and incorporated within phagosomes containing IgG-coated S. typhimurium but not C3 coated S. typhimurium.

Efforts have also been initiated to purify and characterize phagosomes from murine macrophages ingesting yeast or various life cycle stages of Trypanosoma cruzi. Murine macrophages provide a distinct advantage for many of these studies since a number of variants and alternative cell lines exist which have well characterized properties. For example, phagolysosomes are rapidly formed in the J774 cell line, as assessed by incorporation of lysosomal enzyme markers, whereas these markers are missing in compartments purified from P388D1 cells, lacking the mannose-6-phosphate receptor responsible for targeting lysosomal enzymes. Using yeast coated with eosinophil peroxidase, we have shown that plasma membrane receptors, and in particular CR3, form part of the phagosome membrane. Initial experiments with T. cruzi internalization by J774 cells indicate that CR3 is prominently iodinated when eosinophil peroxidase-coated epimastigotes are internalized.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00536-01 LPD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Transcriptional Regulation in Trypanosoma cruzi.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Keith Joiner	Senior Investigator	LPD, NIAID
Others:	G.B. Ogden	Staff Fellow	LPD, NIAID
	B.F. Hall	Medical Staff Fellow	LPD, NIAID

COOPERATING UNITS (if any)

none

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Unit on Microbial Pathogenesis

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.9

PROFESSIONAL:

0.9

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Studies concerning the molecular mechanisms controlling gene expression in Trypanosoma cruzi were initiated. We attempted, in our approach, to identify T. cruzi genes which might share DNA homology with known transcriptional activators in yeast (e.g., GCN4) and mammalian cells (e.g., c-fos and c-myc). We found that T. cruzi DNA, in dot-blot hybridization experiments, hybridizes under stringent conditions with the c-fos- and GCN4-specific probes, but not with a probe specific for c-myc. Further analysis by Southern hybridization revealed that the c-fos-specific probe hybridizes to a single 8.1 kb band in EcoRI- digested T. cruzi DNA. The cloning and sequencing of this 8.1 kb DNA fragment should allow the identification of any c-fos-related genes contained within it. Moreover, if this putative fos-related gene proves to be developmentally regulated, the DNA sequence data may help to identify regions involved in controlling its expression.

LABORATORY OF VIRAL DISEASES
1988 ANNUAL REPORT

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Public Health Service-National Institutes of Health
ANNUAL REPORT OF THE LABORATORY OF VIRAL DISEASES, NIAID

October 1, 1987 to September 30, 1988

Dr. Bernard Moss
Chief, Laboratory of Viral Diseases

RESEARCH OBJECTIVES

The Laboratory of Viral Diseases carries out investigations on the molecular biology of viruses, the interactions of viruses with host cells, the pathogenesis of viral diseases, and host defense mechanisms that are designed to increase fundamental knowledge and facilitate the development of new approaches to the prevention and treatment of virus infections. Current topics of basic research include: regulation of gene expression, mechanisms of DNA replication, assembly and transport of virus particles, virus growth factors, determinants of virus virulence, host resistance genes, and viral targets of humoral and cell mediated immunity. More applied areas of investigation include the development of recombinant expression vectors, candidate vaccines, and antiviral agents. These studies involve a wide range of viruses including human immunodeficiency virus.

REGULATION OF VIRUS GENE EXPRESSION

DNA viruses provide model systems for studying basic aspects of gene expression and can be used as expression vectors for a variety of purposes. Several different virus systems are currently under investigation by members of LVD.

POXVIRUSES

Poxviruses are large DNA viruses that replicate in the cytoplasm of infected cells. In order to express and replicate their genomes outside of the nucleus, poxviruses must encode enzymes for these processes. This feature makes poxviruses, among which vaccinia virus is the prototype, unique for studying the regulation of transcription. Additional information regarding transcription is also needed to improve vaccinia virus expression vectors which are currently being tested as candidate live vaccines against several diseases including AIDS. During the past year progress has been made in determining the signals used for initiation and termination of transcription of early and late vaccinia virus genes and the enzymes and factors needed to carry out these steps.

Purification and characterization of a factor required for transcription of vaccinia virus early genes. The inability of highly purified vaccinia virus RNA polymerase to transcribe vaccinia virus genes led to our discovery of a specific transcription factor. A complementation assay was developed to quantitate the recovery of the factor during purification. An observation that the factor binds specifically to early vaccinia virus promoters was exploited in the final affinity chromatography step. The purified protein, which we have named VETF, has a molecular weight of approximately 150,000 and contains two polypeptides of 77,000 and 82,000. The purified factor stimulates transcription from all early promoters tested, binds specifically to early promoters, and exhibits a DNA-dependent ATPase activity. The latter may explain the previously described requirement for ATP hydrolysis in transcription. We suspect that VETF is the functional equivalent of the eukaryotic TATA binding factor which has not yet been purified. (Broyles, Shuman and Moss)

Factor-dependent transcription termination by vaccinia RNA polymerase. The mechanism used for termination of mRNA in eukaryotic cells is not yet known. Transcription termination by vaccinia RNA polymerase is dependent on a trans-acting factor that we have named VTF and which is associated with the vaccinia capping enzyme. VTF induces termination approximately 50 nucleotides downstream of a signal sequence TTTTNT in the template strand. Since the signal is transcribed, we carried out experiments to determine the role of the nascent RNA in termination. We found that halogenated analogs of UTP specifically abrogated factor-dependent termination without affecting the synthesis of read-through transcripts. These results indicate that the cis-acting termination signal is recognized in the nascent RNA rather than the template DNA. (Shuman and Moss).

Late vaccinia virus mRNA has capped poly(A) leaders of variable length. Several research groups have recently obtained evidence for a novel poly(A) structure at the 5' ends of vaccinia virus late mRNAs. We have demonstrated capped poly(A) leaders of variable length located immediately upstream of the translation initiation codon by direct analysis of a major late RNA species. A "decapping-recapping" protocol was used to specifically substitute a radioactively labeled phosphate for an unlabeled one within the cap structure. Sequences of the type m⁷G(5')pppAmp(Ap)nUpG- where N varies from a few to more than 40 nucleotides was deduced by analysis of the length and sequence of RNase H, T1 and U2 digestion products. The function of the poly(A) leader is not yet known. (Ahn and Moss)

In vitro synthesis of vaccinia virus mRNA containing a 5' poly(A) leader. In order to understand the mechanism of expression of vaccinia virus late mRNAs, an in vitro transcription system was developed from virus infected HeLa cells. Extracts made at 6 or more hours after vaccinia virus infection were able to transcribe duplex DNA templates containing a vaccinia virus late gene. Mutations in the conserved TAAAT sequence led to inactivation of the late promoter. Analysis of the transcripts revealed that the poly(A) leader was synthesized. A cis-splicing mechanism of poly(A) addition was ruled out. Three models of poly(A) leader formation are currently being tested: RNA polymerase initiates synthesis with a run of adenylate residues; a poly(A) primer is used for initiation; the poly(A) leader is attached by trans-ligation. (Wright and Moss)

Characterization of the specificity of vaccinia virus poly(A) polymerase for nucleotides and nucleotide analogs. Eukaryotic and viral mRNAs generally have a 3' poly(A) tail. The enzyme responsible for this addition to early vaccinia virus mRNAs was previously purified from virus particles. The selectivity of this enzyme for ATP was analyzed. We found that UTP, GTP, or CTP could be utilized although at much lower rates than ATP. In the presence of ATP, however, incorporation of other nucleotides was not detected. Kinetic analysis of poly(A) synthesis indicated that it is a biphasic reaction. (Shuman and Moss)

Identification of a vaccinia virus gene encoding a type I DNA topoisomerase and expression in *Escherichia coli*. Type I DNA topoisomerases are found in both prokaryotic and eukaryotic organisms. A topoisomerase with a molecular weight of 32,000 present in vaccinia virus particles was purified to homogeneity and the NH₂-terminal sequence was determined. This sequence matched an open-reading-frame present in the vaccinia virus genome proving that the protein is virus-encoded. The gene was expressed in an *E. coli* vector and large amounts of active topoisomerase I was obtained. Unlike cellular topoisomerase I, the viral enzyme is resistant to camptothecin. (Shuman and Moss)

PARVOVIRUSES

Adeno-associated virus (AAV), a defective member of the parvovirus family, is dependent on co-infection with a member of the adenovirus or herpesvirus family. AAV gene expression appears to be dependent on interactions involving cellular and helper virus products.

Regulation of expression of AAV structural proteins. AAV virions contain three structural proteins, A, B, and C. Continued studies during the past year support a mechanism in which the use of a novel translation initiation codon, ACG, is used to express the B protein and alternative splicing is required for expression of the A protein. (Muralidhar, Koczot, Fabish, Rose)

Regulation of expression of AAV non-structural proteins. At least six non-structural proteins have been identified by *in vitro* translation of AAV transcripts and/or Western blotting of infected cell lysates using antibodies produced against synthetic peptides. Current studies indicate that the unspliced p5 and p19 transcripts express the 70 and 50 kDa non-structural proteins and that spliced p5 and p19 transcripts generate the 60 and 42 kDa non-structural proteins. Based on reactivity with antisera to amino-terminal peptides, it can be concluded that the 70, 50, and 42 kDa proteins are initiated from the first AUG of their respective mRNAs. (Sebring, Wong, Muralidhar, and Rose).

VIRUS DNA REPLICATION

Viruses provide useful systems for analyzing the diversity of mechanisms employed in DNA replication. In addition, the virus encoded factors provide potential targets for chemotherapy.

REPLICATION OF VACCINIA VIRUS DNA

Poxviruses provide a unique experimental system for studying DNA replication since required enzymes and factors are encoded with the virus genome and replication occurs in the cytoplasm.

Resolution of vaccinia virus concatemer junctions. Previous studies have indicated that large DNA concatemers form during the replication of vaccinia virus DNA. The concatemer junction was cloned in an *E. coli* plasmid and a transfection assay for resolution of the junction in vaccinia virus infected cells was developed. The introduction of a series of symmetrical insertions, deletions, and nucleotide substitutions into the DNA revealed that both a palindromic structure and specific 20 nucleotide sequence are required for resolution. A model for resolution involving site-specific recombination and oriented branch migration is consistent with the data. (Merchlinsky and Moss)

A late vaccinia virus protein is required for resolution of concatemer junctions. As a first step in determining the protein factors required for resolution of vaccinia virus DNA concatemers, a series of conditionally lethal temperature sensitive vaccinia virus mutants were screened. Three phenotypes were observed: mutants that are DNA replication negative; mutants that replicate DNA and process the concatemer junction in a normal manner; and mutants that replicate DNA but fail to process the concatemer junction. The latter group were all defective in late protein synthesis indicating that a late viral function is required for resolution. (Merchlinsky and Moss)

Intramolecular homologous recombination in vaccinia virus infected cells. Intramolecular homologous recombination was analyzed by transfecting plasmids into

vaccinia virus infected cells. Recombination was monitored in cells infected with each of 32 complementation groups of vaccinia virus thermolabile mutants at the non-permissive temperature. Three phenotypes were observed: the majority, which included viruses defective in late protein synthesis, carried out recombination efficiently; the DNA replication negative group did not replicate or recombine the plasmids; the third group which consisted of only a single mutant replicated virion DNA and resolved concatemer junctions but did not replicate or recombine plasmid DNA. These results distinguish between resolution, a site specific recombination process which requires synthesis of late vaccinia proteins, and intramolecular homologous recombination which does not. (Merchlinsky)

Drug-resistance domains of vaccinia virus DNA polymerase. Previous work established that an aphidicolin-resistant vaccinia virus DNA polymerase contains a single mutation resulting in a substitution of methionine for leucine. During the past year, methods of site directed mutagenesis have been optimized in order to finely map the aphidicolin resistance domain and determine structure-function relationships. (DeFilippes)

REPLICATION OF ADENO-ASSOCIATED VIRUS DNA

The AAV genome consists of either a plus or a minus linear single-stranded DNA with partially palindromic inverted terminal repeats. Previous studies from this laboratory established that replication of AAV DNA is initiated on single-stranded genomic templates and proceeds by a self-priming mechanism with the 3'-terminal hairpin serving as the primer.

Proteins associated with the terminal palindromes. To identify the factors responsible for site-specific processing of the AAV genome, DNA structures and associated proteins were released from purified virions with 4 M guanidinium chloride. Structures corresponding to terminal palindromic fragments were identified and two proteins were found to be associated with genomic length strands of DNA. The data suggest that terminal palindromic sequences contain a packaging signal and that processing occurs at a single site. (Chatterjee, Sebring, and Rose)

REPLICATION OF HERPES SIMPLEX VIRUS (HSV) DNA

HSV and other members of the herpesvirus family are important human pathogens. The study of HSV DNA synthesis is useful both as a model for eukaryotic DNA replication and for designing new strategies for therapy. Previous work from this laboratory demonstrated that seven HSV genes are necessary and sufficient for authentic origin-dependent DNA replication. During the past year progress has been made in identifying the products of these genes.

Identification of the products of the genes required for HSV DNA replication. As a first step toward a biochemical characterization of HSV DNA replication, we set out to identify the products of all of the essential replication genes. Toward this goal we raised rabbit antisera against the products of each of these seven genes including the three known replication proteins. The immunogens used were either synthetic peptides or fusion proteins expressed in *E. coli*. These sera were then used to analyze the expression of the seven replication proteins during viral infection. The sera directed against previously identified HSV replication proteins (polymerase, ICP8, and UL42) provided us with a useful frame of reference during our analysis of the other four gene products.

Using these reagents we have now identified the products of *UL5*, *UL8*, *UL9*, and

UL52 in HSV infected cells. The apparent molecular weight of each polypeptide is close to that predicted from DNA sequence analysis. As expected for proteins involved in viral DNA synthesis, these proteins all localize to the nucleus, and they are expressed in a manner consistent with that of the products of delayed early genes. In addition, our data suggest that these four proteins are made in very low amounts relative to the other three proteins (ICP8, polymerase, and UL42). (Olivo and Challberg)

Expression of HSV proteins in insect cells. Primarily to overcome problems related to their low abundance, we have expressed *UL5*, *UL8*, *UL9*, and *UL52* in insect cells using the baculovirus expression system. The proteins made in insect cells are also approximately of the predicted size, and in fact are indistinguishable from the corresponding protein found in HSV infected vero cells. All of our results, therefore, support the interpretation of the HSV DNA sequence proposed by our collaborator, Dr. Duncan McGeoch, of the Institute of Virology in Glasgow. In addition, our antisera and recombinant baculoviruses have become extremely useful in reagents in our biochemical studies of HSV DNA replication. (Olivo, Nelson, and Challberg)

Identification of the HSV gene encoding an origin binding protein. By analogy with better characterized prokaryotic and eukaryotic DNA replication systems, it seems likely that the primary event in the initiation of HSV DNA replication involves the binding of one or more proteins to sequences within *ori*, and/or *ori_L*. An origin-specific DNA binding activity in fact has been identified in extracts of HSV-infected cells, although the involvement of this activity in HSV DNA replication has not been demonstrated. We have made use of our specific antisera and recombinant baculoviruses to identify the HSV gene encoding this origin binding protein. Using immunoassay for protein-DNA interaction, we have shown that UL9 protein binds specifically to the HSV origins of DNA replication. DNase I footprint analysis has shown that the UL9 protein interacts with two related sites on *oris*, located on each arm of a nearly perfect palindrome. Our data strongly suggest that the origin binding activity described previously is the product of the UL9 gene. (Olivo, Nelson, and Challberg)

Homologous recombination in HSV-infected cells. HSV DNA, or other DNAs introduced into HSV-infected cells, undergo high levels of homologous recombination. In collaboration with Drs. Peter Weber and Joseph Glorioso of the University of Michigan, we have begun to characterize this process. In particular, we have shown (1) that extensive recombination requires replication of the target DNA by the HSV replication system, and (2) that the seven genes that are sufficient for DNA replication also suffice for recombination. Our data thus strongly suggest that the process of HSV DNA replication itself is recombinogenic. (Challberg)

FOLDING, ASSEMBLY AND TRANSPORT OF VIRAL GLYCOPROTEINS

Understanding the folding, assembly, and transport of viral glycoproteins is of general interest and practical importance since it could lead to a greater understanding of antigenic structure. This information is critical for designing vaccines that induce neutralizing antibody.

Folding of the influenza hemagglutinin (HA) occurs rapidly after synthesis. HA oligomerization begins 1 to 2 minutes following synthesis. Under normal circumstances this is first detected in post-endoplasmic reticulum compartments. However, when cells are incubated with Brefeldin A, a fungal metabolite which prevents transport to the golgi, trimerization occurs in the endoplasmic reticulum. (Yewdell)

VIRUS HOST INTERACTIONS

Molecular virology studies have largely focused on the virus itself. The importance of virus/host interactions in determining viral pathogenesis and host immunity is now receiving greater emphasis in LVD.

Vaccinia virus encodes a gene that is a member of the complement binding superfamily. A protein that is secreted from vaccinia virus infected cells was purified and the NH₂-terminal sequence was determined. Based on this sequence, the open-reading-frame encoding the protein was located on the vaccinia virus genome. Remarkably, the sequence indicated that the vaccinia protein was a member of a family of complement binding proteins and was very similar in structure and sequence to C4b. The ability of a virus to negatively regulate the complement cascade could help counteract host immune defenses. (Kotwal and Moss)

Vaccinia virus encodes two proteins that are structurally related to members of the plasma serine protease superfamily. Nucleotide sequencing has revealed that vaccinia virus contains 2 genes that belong to the eukaryotic super family of plasma serine protease inhibitors. The 2 proteins, called SPI-1 and SPI-2, have a 44% amino acid identity with each other and differ in the active site region suggesting that they interact with different serine proteases. SPI-2 is homologous to a cowpox virus protein that has a role in the formation of hemorrhagic pocks. These protease inhibitors also may be involved in protection against host defenses. (Kotwal and Moss)

Mutation of the cowpox virus SPI-2 gene. The gene encoding the SPI-2 gene of cowpox virus was mutated by recombinant DNA techniques. In contrast to the wild-type virus which makes hemorrhagic pocks, the mutant makes white pocks in the chicken embryo chorioallantoic membrane. Histochemical examination reveals alterations in the influx of heterophils into the pock. (Palumbo and Buller)

Evidence for a new vaccinia virus growth factor. Virus-free, concentrated culture supernatants of vaccinia virus infected cells specifically stimulates the proliferation of adherent macrophages from human peripheral blood cells. Efforts to determine whether this action occurs through immune or non-immune mechanisms are in progress. (Buller and Palumbo)

Genetics of innate resistance to ectromelia virus. One strain of mice, C57BL/6J, is highly susceptible to ectromelia infection whereas another, A/J is not. Sister:brother cross-breeding experiments are being carried out to identify the resistance gene(s). Thus far, after 4 generations, recombinant mice that are resistance to severe disease are still present. At least 8 cycles will be carried out before testing for congenicity of the resistance gene(s). (Buller).

VIRUS EXPRESSION VECTORS

The use of expression vectors has become an important part of recombinant DNA technology. Members of LVD have been instrumental in developing vaccinia virus as an expression vector and employing it for vaccine research and as a candidate live vaccine.

New method developed for dominant selection of recombinant vaccinia viruses. The *E. coli* gpt gene was used to provide dominant selection for vaccinia virus expression vectors. By synthesizing xanthine-guanine phosphoribosyltransferase, only recombinant vaccinia viruses can form plaques in the presence of selective medium

containing mycophenolic acid, xanthine and hypoxanthine. To use the system efficiently, a new set of vectors containing a strong late promoter with polylinker insertion sites in all three reading frames was constructed. (Falkner and Moss)

Use of a carrier protein to transport peptide sequences to the cell surface. The immunogenicity of proteins may be enhanced by their expression on the surface of cells. Previous attempts to construct hybrid proteins that can be used to transport peptide sequences to the plasma membrane have been generally unsuccessful because of perturbations in protein folding. In seeking to minimize this problem, we have employed the less common type of integral membrane protein which has an uncleaved signal-anchor domain and an extracellular carboxyl portion. To test the model, we expressed hybrid proteins containing N-terminal portions of the respiratory syncytial virus glycoprotein fused to the dominant repeating epitope of the circumsporozoite protein of *Plasmodium falciparum*. Surface expression in tissue culture and enhanced immunogenicity in experimental animals was achieved. (Satchidanandan and Moss)

Prevention of vaccinia virus lethal infection in immunodeficient mice by vector directed lymphokine expression. The potential for vaccinia virus to produce a progressive infection in immunodeficient individuals is a serious problem for the future use of live recombinant vaccinia virus as a vaccine. We have demonstrated that immunodeficient athymic nude mice are subject to lethal disseminated infections with vaccinia virus strain WR. However, when the gene for human interleukin 2 was inserted into vaccinia virus, the nude mice were able to control and eliminate the infection. Recombinant vaccinia virus that express IL-2 are also more attenuated for normal mice, however, this does not seem to impair their immunogenicity to a significant degree. (Flexner, Huglin, and Moss)

Protection of mice against HSV infection by immunization with recombinant vaccinia viruses expressing HSV gD or gB. Significant protection of mice against lethal and latent HSV infection lasted for more than one year after a single intradermal vaccination with a recombinant vaccinia virus expressing HSV gD. Protection has also been obtained with recombinant virus expressing HSV gB, however the duration of this immunity has not yet been determined. These data suggest that both genes would be useful in a recombinant vaccine. (Moss and collaborators)

Successful vaccination against HSV and influenza with a live polyvalent vaccinia vector. A recombinant vaccinia virus that expresses both the influenza hemagglutinin and the HSV gD genes was constructed. Mice inoculated intradermally with this vaccine were protected against both systemic HSV infection and lower respiratory influenza infection. Prior immunity to either HSV or influenza virus apparently did not affect the replication of the double recombinant or influence protection against either virus. (Flexner, Moss, and collaborators)

NEW INFECTIOUS AGENTS

HUMAN IMMUNODEFICIENCY VIRUS - AIDS

The continued spread of human immunodeficiency virus (HIV) makes it imperative to develop vaccines and therapeutic agents to prevent further infection and help those with AIDS. In LVD, recombinant vaccinia virus expression vectors have been employed to determine the immune response to HIV and to develop candidate vaccines. The interaction of HIV with its surface receptor, CD4, has been studied in order to design new therapeutic agents.

Targets of humoral and cell mediated immunity. Recombinant vaccinia viruses that express HIV envelope gp160, gag, reverse transcriptase, and tat genes have been constructed and characterized. Immunization of macaques with recombinant vaccinia virus containing the HIV gp160 gene led to the production of isolate specific neutralizing antibody and a group specific cell mediated immune response. The targets of cytotoxic T cells (CTL) have been determined in vaccinated mice and in humans naturally infected with HIV. Following vaccination with the gp160 recombinant, spleen cells from mice were restimulated *in vitro* with a transfected histocompatible cell line expressing the same gene and then tested for CTL activity. These studies showed that H-2d mice are high responders and H-2k mice are low responders. Moreover, the H-2d mice respond predominantly to a single immunodominant site represented by a 15-residue synthetic peptide from a highly variable region of gp120. Studies in humans naturally infected with HIV, however, were quite different. Recombinant vaccinia viruses expressing HIV genes were used to infect autologous cells to make CTL targets. HIV seropositive individuals were found to have circulating CTL with group specificity to gp160, gag, and reverse transcriptase. These data suggest that incorporation of internal as well as envelope antigens might be useful in a vaccine. (Earl, Flexner, Moss and collaborators)

Location of the HIV binding site within the CD4 receptor. A soluble recombinant polypeptide comprising the amino-terminal half of the extracellular region of the CD4 molecule was expressed using a vaccinia virus vector system. Epitope analysis with a panel of anti-CD4 murine monoclonal antibodies indicated that the 178 amino acid CD4 fragment reacted with those antibodies known to block the interaction between CD4 and the HIV glycoprotein but reacted poorly or not at all with those antibodies that do not block this interaction. Furthermore, the 178 amino acid molecule formed a specific complex with gp120. (Berger and Moss)

Selective killing of HIV-infected cells by a recombinant human CD4-pseudomonas exotoxin hybrid protein. A hybrid protein consisting of 178 amino acids of CD4 and the second and third domains of *Pseudomonas* exotoxin A was made in collaboration with investigators in NCI. The hybrid protein was shown to bind to gp120 and to selectively kill cells infected with HIV. Studies with recombinant vaccinia viruses demonstrated that the killing is due to expression of the HIV envelope protein. This toxin is being evaluated as a possible therapeutic agent for the treatment of AIDS patients. (Berger, Mizukami, Fuerst, Moss and collaborators)

HUMAN HERPESVIRUS 6

Human herpesvirus 6 (HHV-6) is a new member of the herpesvirus group first isolated in 1986. Current data suggests that the virus is ubiquitous in humans and that infection occurs in early childhood. There is evidence that HHV-6 may be the causative agent of exanthem subitum (roseola infantum), a common disease of infants. Isolation of the virus from AIDS patients as well as normal individuals suggests that the virus establishes a latent state. It is unknown whether the virus causes other diseases during primary or recurrent infections.

HHV-6 propagation. Significant advances were made in the methodology for virus propagation and high titer stocks have now been made. (Frenkel and Katsafanos).

Characterization of the viral genome and cloning of viral DNA. Cleavage patterns for 20 enzymes have been obtained and the genome has been estimated as 120 kilobase pairs. Approximately 40% of the genome has been cloned. (Steele, DiLuca and Frenkel).

Virus tropism and interaction with host cells. Evidence for T cell tropism has been obtained. HHV-6 replication requires T cell activation which may be mediated through the CD3 pathway. (Frenkel and collaborators)

ADMINISTRATIVE CHANGES

During the past year the phase 1 renovations at Twinbrook 2 were completed and research laboratories were occupied by Drs. Bennink, Frenkel, Yewdell and their associates. The renovations in building 4 are nearing completion and a move from building 5 is imminent. As usual, there has been a healthy turnover of young investigators who came to LVD to further their research training. Some have gone on to academic appointments and others to positions in industry.

HONORS AND AWARDS

Dr. Buller received the NIH Award of Merit. Dr. Moss was inducted into the National Academy of Sciences, was a Wellcome Visiting Professor in Microbiology at the University of Florida and gave several distinguished lectures including the Charles A. Stuart Memorial Lecture at Brown University, the Boyce Thompson Distinguished Lecture at Cornell University, and the Cancer Research Campaign Lecture at the University of Glasgow.

Members of LVD serve on the editorial boards of numerous scientific journals. Dr. Rose was renominated to the editorial board of the *Journal of Virology*; Dr. Levy continues to serve on the boards of the *Journal of Bioactive Polymers* and the *Journal of Biological Regulators and Homeostatic Agents*; Dr. Moss is an associate editor of *Virology* and is on the boards of the *Journal of Virology* and *Advances in Virus Research*.

Members of LVD presented numerous lectures and seminars in this country and abroad.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00020-13 LVD
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies on the Treatment of Disease with the Interferon System		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Hilton B. Levy Section Head LVD, NIAID		
COOPERATING UNITS (if any) Dr. Andre Salazar, Walter Reed Army Medical Research Institute; Dr. Samuel Baron, U. of Texas Medical School, Galveston, Texas; Dr. Christopher Bever, Johns Hopkins Med. School, Balt., MD; Dr. Meir Kende, USAMRIID, Frederick, MD.		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Molecular Virology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 1	PROFESSIONAL 1	OTHER 0
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☐ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The primate- effective interferon inducer, poly inosinic-polycytidylic acid complexed with poly l-lysine and carboxymethyl cellulose, polyICLC, administered intravenously, has been shown to induce changes in some subsets of white blood cells and in cell-associated immune functions, in monkeys and man. In some instances the effect is dose related, in that low doses can cause one effect, while higher doses will cause the opposite effect. At low doses there is an increase in percent lymphocytes in the blood and an increase in NK cell activity, while at higher doses there is a decrease in both of these parameters. At the higher doses there is, however, a marked increase in total WBC, all of which is attributable to an increase in granulocytes. At all doses tested there is an increase in macrophage numbers and activity, and an increase in 2'5' oligo A synthetase. The expression of DR+ antigen is also enhanced. The ratio of T4 to T8 lymphocytes is increased, and this effect can be elicited each time on reinjection. The effects are transient, lasting up to 3 days. A protocol to treat AIDS patients with polyICLC has been developed and approved by FDA.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-AI-00123-22-LVD						
PERIOD COVERED October 1, 1987 to September 30, 1988								
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Structure and Replication of Poxvirus DNA								
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation) <table style="width: 100%; margin-top: 10px;"> <tr> <td style="width: 33%;">PI: M. Merchlinsky</td> <td style="width: 33%;">Staff Fellow</td> <td style="width: 33%;">LVD, NIAID</td> </tr> <tr> <td>Others: B. Moss</td> <td>Chief</td> <td>LVD, NIAID</td> </tr> </table>			PI: M. Merchlinsky	Staff Fellow	LVD, NIAID	Others: B. Moss	Chief	LVD, NIAID
PI: M. Merchlinsky	Staff Fellow	LVD, NIAID						
Others: B. Moss	Chief	LVD, NIAID						
COOPERATING UNITS (if any)								
LAB/BRANCH Laboratory of Viral Diseases								
SECTION Genetic Engineering Section								
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892								
TOTAL MAN-YEARS 1.3	PROFESSIONAL 1.1	OTHER: 0.2						
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews								
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) Poxviruses provide a unique experimental system for studying DNA replication. Required enzymes and factors are encoded within the viral genome and DNA synthesis and processing occurs within the cytoplasmic compartment of the cell. Analysis of a large series of temperature sensitive mutants of vaccinia virus has allowed us to divide genome formation into two stages: DNA replication which generates concatemers and resolution of concatemers into monomers. Expression of early genes is required for the former and late genes for the latter. Site-directed mutagenesis was used to define the sequence required for the resolution of the vaccinia virus concatemer junction. A model for resolution involving site-specific recombination and oriented branch migration is consistent with our data. The requirements for resolution and intramolecular recombination are different. Biochemical and genetic studies indicated that early functions including DNA replication are necessary for intramolecular recombination in transfected plasmids but that late functions are not necessary. A DNA⁺ temperature sensitive mutant blocked in recombination was found.								

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00126-15-LVD
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Functional Analysis of Vaccinia Virus DNA		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: F. M. DeFilippes Research Physicist LVD, NIAID		
COOPERATING UNITS (if any)		
LAB. BRANCH Laboratory of Viral Diseases		
SECTION Macromolecular Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS 1.1	PROFESSIONAL 1.0	OTHER 0.1
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☐ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) A vaccinia virus mutant which is resistant to aphidicolin (AP) codes for an AP-resistant DNA polymerase. A change from wild-type (WT) cytosine to adenine at position 2430 of the DNA sequence changes leucine to methionine in the polymerase and confers AP resistance. The mutation site is in a region of six amino acids which are conserved in several DNA polymerases. I am substituting other amino acids for the WT leucine at the original mutation site and testing for AP resistance. To proceed efficiently, I have developed the technique for site-directed mutagenesis described by Eckstein. This procedure produces recombinant single stranded (ss) DNA containing a mutation which is specified in an oligonucleotide primer. Frequently over 75% of the plaques produced by transformation contain the desired alteration so that a preliminary screen for the mutation is unnecessary. Also, one can anneal different primers with different substituted nucleotides to a single template. One reaction used three primers with different substitutions and only five plaques had to be picked to obtain the three mutations. Furthermore, sequence permutations over a short region can be amplified by annealing a single primer with substitutions at positions starting near one end and extending to the center of the oligonucleotide. Because centrally located alterations are obtained more frequently than peripheral ones, different plaques contain different sequence alterations. Also, the marker transfer reaction was modified to use ss recombinants rather than the usual plasmid (ds) DNA. Eight micrograms of ss DNA produced as many mutant plaques as one microgram of ds DNA. Finally, the template used for one set of AP mutagenic reactions contained the phosphonoacetate (PA) resistance mutation. This construction can be used to indicate whether or not a given substitution at the AP site is liable to be lethal since the recombinant can be tested for PA resistance. The results so far indicate that substitution of leucine by proline, arginine, tryptophan or glutamic acid does not confer AP resistance.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZOI AI-00294-07-LVD
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (20 characters or less. Title must fit on one line between the borders.) Structure and Function of Adenovirus DNA		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: J. A. Rose Section Head LVD, NIAID		
COOPERATING UNITS (if any) R. McPherson, M.D., Department of Pathology, Georgetown University Hospital, Washington, DC; D. Klessing, Waksman Institute, Piscataway, NJ		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Molecular Structure Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 0.4	PROFESSIONAL: 0.3	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Among objectives of these studies has been the application of physical, biochemical, and biological techniques to characterize the structure and function of certain sequences (e.g., the inverted terminal repeats) and genes of adenovirus (Ad) DNA. In earlier studies, we first identified and characterized the <u>VA RNA gene/transcript</u> and inverted terminal repeats in Ad DNA. We have continued to investigate the specific functions of several early Ad genes, e.g., the <u>VA and DNA-binding protein genes</u>. Our results indicate that these latter genes are involved in the <u>regulation of translation</u> of certain viral mRNAs. Among methods used are <u>gradient sedimentation</u>, <u>DNA cleavage with restriction endonucleases</u>, <u>gel electrophoresis</u>, <u>base sequence analysis</u>, and <u>DNA transfection</u>.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (90 characters or less. Title must fit on one line between the borders.)

Helper Factors Required for Expression of the Adeno-Associated Virus Genome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. A. Rose

Section Head

LVD, NIAID

COOPERATING UNITS (if any)

M. Challberg, Senior Staff Fellow, LVD, NIAID; L. Mishra, Senior Staff Fellow, Division of Blood and Blood Products, CBER, FDA

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

1.25

PROFESSIONAL

1.25

OTHER

CHECK APPROPRIATE BOXES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unenriched type. Do not exceed the space provided.)

The main objectives of this project are (i) to define where and how each required helper virus factor regulates expression of defective human paravovirus (AAV) genomes, and (ii) to relate these findings to their respective roles in the replication of the helper viruses (adenoviruses, herpesvirus) themselves as well as to potentials for selective interference with viral infection. We previously mapped the adenovirus genes required for AAV replication and continue to investigate their specific helper functions. Similar studies are in progress with herpes simplex viruses. Among methods used are specific immunofluorescence, cleavage of DNA with restriction endonucleases, DNA cloning, gel electrophoresis, blot-hybridization analyses, and DNA transfection of both simian and human cell lines.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-AI-00296-07-LVD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization and Production of Parvovirus Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	J. A. Rose	Section Head	LVD, NIAID
Others:	E. Sebring	Research Chemist	LVD, NIAID
	K. Wong	Medical Staff Fellow	LVD, NIAID
	S. Muralidhar	Visiting Fellow	LVD, NIAID

COOPERATING UNITS (if any)

C. W. Anderson, Brookhaven National Laboratory, Upton, NY 11973

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

4.6

PROFESSIONAL:

3.1

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The main objective of these studies are (1) to identify and characterize all proteins that are specified by the defective human parvoviruses (AAV) and to determine similarities and differences with autonomous parvovirus proteins, (2) to define the mechanism(s) by which the AAV proteins arise, and (3) to define specific functions of the AAV proteins. We have identified several AAV non-structural proteins which were previously undetected. At least one of these proteins is necessary for viral DNA replication. Post-translational processing does not account for production of any AAV structural proteins although they share large segments of sequences-in-common. It is now clear, however, that these proteins originate from independent in-frame initiations. Mechanisms that regulate expression of AAV proteins are of fundamental interest, and we have now shown that one AAV structural protein is initiated by a codon not known previously to act as an initiation codon in higher eukaryotes. Furthermore, our current findings (i) support a "scanning mechanism" in the translational expression of polycistronic eukaryotic mRNAs, and (ii) demonstrate that alternative mRNA splicing is required for effective translational expression of the largest AAV capsid protein. Among methods used are site-directed mutagenesis, affinity chromatography, gel electrophoresis, in vitro translation of viral RNA, electrophoretic and HPLC analyses of V8 protease, and tryptic peptides and aminoterminal sequencing of purified polypeptides.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT 80 characters or less Title must fit on one line between the borders

Mechanism and Regulation of Adeno-associated Virus DNA Replication

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)

PI: J. A. Rose

Section Head

LVD, NIAID

Others: E. Sebring

Research Chemist

LVD, NIAID

S. Chatterjee-Das

Visiting Associate

LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

2.0

PROFESSIONAL

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The primary objective of this project is to define molecular and biochemical mechanisms involved in eukaryotic DNA synthesis. To approach this problem, we are investigating adeno-associated virus (AAV) DNA replication in both *in vivo* and *in vitro* systems. We have shown that AAV DNA synthesis can be initiated *in vitro* in cell-free extracts and that replicating forms that correspond to those identified *in vivo* can be synthesized with either endogenous or exogenously added templates. Recently, we have identified two DNA-associated proteins that may play an important role in the required processing of self-primed unit length hairpin and concatemeric intermediates of DNA replication. The observed mode of AAV DNA replication may serve as a model for telomere replication in eukaryotes. Among methods used are affinity chromatography, gel-electrophoresis, DNA sequence analysis, and restriction cleavage of DNA molecules.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI-00298-07-LVD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Vaccinia Virus as an Expression Vector

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	B. Moss	Laboratory Chief	LVD, NIAID
	C. Flexner	Medical Staff Fellow	LVD, NIAID
	T. Fuerst	Guest Researcher	LVD, NIAID
	A. Hugin	Visiting Fellow	LVD, NIAID
	V. Satchidanandan	Guest Researcher	LVD, NIAID

COOPERATING UNITS (if any)

LID, NIAID; LPD, NIAID; New York University School of Medicine

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.7

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Vaccinia virus has been developed into a eukaryotic expression vector. A chimeric gene is formed by ligating vaccinia virus transcriptional regulatory signals to a foreign protein coding sequence. Homologous recombination is used to insert the chimeric gene into a non-essential region of the vaccinia virus genome. During the past year, we have used stronger late promoters to increase the level of expression and develop a new dominant selection system, based on co-expression of the *Escherichia coli gpt* gene, for isolation of recombinant viruses. The respiratory syncytial virus glycoprotein B gene was employed to construct hybrid genes in order to bring peptide sequences to the cell surface and enhance immunogenicity. The athymic immunodeficient nude mouse was developed as a model for progressive infection with vaccinia virus. We found that the pathogenicity of vaccinia virus for nude mice was abrogated by expression of the lymphokine IL-2. Continued work using a recombinant vaccinia virus that expresses the herpes simplex virus type 1 (HSV-1) glycoprotein D gene for vaccination of mice, established that protective immunity against lethal and latent infections lasted for more than a year, that immunity could be boosted with a second vaccination, but that pre-existing immunity to vaccinia virus was detrimental. Protective immunity to HSV-1 was also induced with a recombinant vaccinia virus that expressed the gB gene. Polyvalent expression vectors were constructed that protected mice against both HSV-1 and influenza virus. In addition, recombinant vaccinia virus was used to determine influenza, HSV-1, and malaria targets of cytotoxic T cells.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. The title is on one line between the corners)
Pathogenesis of Orthopoxvirus Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator; (Name, title, laboratory and institute affiliation)

PI: R.M.L. Buller Visiting Scientist LVD, NIAID
Others: C. Duarte Bio. Lab. Tech. (Micro) LVD, NIAID
G. Palumbo IRTA Fellow LVD, NIAID

COOPERATING UNITS (if any)

LIP, NIAID (H.C. Morse, III); Cancer Immunology Branch, NCI (Dr. G. Shearer, Dr. A. Singer, Dr. T. Mizuochi); U. of Connecticut (T. Fredrickson); Dr. D. Pickup, Duke University.

LAB/BRANCH

Laboratory of Viral Disease

SECTION

Macromolecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

2.9

PROFESSIONAL

1.9

OTHER:

1.0

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In this project we focused our studies on the genetic basis both of orthopoxvirus virulence and of host resistance to virus infection. The acquired knowledge should contribute towards the development of safe, effective, recombinant vaccinia virus vaccines for animal and human use. A deletion mutant of cowpox virus has been constructed which lacks a functional gene for a 38 kilodalton protein involved in the generation of a hemorrhagic pock on the chorioallantoic membrane of the chicken embryo. The mutant pock contains significantly more heterophils than the WT, and the significance of this observation is being investigated. An activity from vaccinia virus infected culture supernatants has been identified that causes ³H-thymidine incorporation into DNA of cells from human peripheral blood lymphocyte populations. Experiments are ongoing to determine whether the activity is virus-coded and the mechanism of action on the target cell(s). Using the cross-intercross system, the A strain of mouse (disease susceptible) was crossed with a disease resistant C57BL/6J mouse in order to generate a mouse strain A.B6-Rmp (resistance to mousepox) congenic for the resistance loci. This will aid in studying the non-H2 innate resistance genes important in resistance to disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00307-07-LVD
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Regulation of Vaccinia Virus Gene Expression		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	B. Moss	Laboratory Chief
Others:	A. J. Davidson	Visiting Associate
	B. Ahn	Visiting Fellow
	B. Amegadzie	Visiting Fellow
	S. Broyles	Staff Fellow
	S. Shuman	Medical Staff Fellow
	C. Wright	Guest Researcher
		LVD, NIAID LVD, NIAID LVD, NIAID LVD, NIAID LVD, NIAID LVD, NIAID
COOPERATING UNITS (if any)		
None		
LAB/BRANCH		
Laboratory of Viral Diseases		
SECTION		
Genetic Engineering Section		
INSTITUTE AND LOCATION		
NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.2	4.0	1.2
CHECK APPROPRIATE BOX(ES)		
☐ (a) Human subjects	☐ (b) Human tissues	☒ (c) Neither
☐ (a1) Minors		
☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
Vaccinia virus has a genome of about 85,000 base pairs that encodes approximately 200 polypeptides. These genes are expressed within the cytoplasm in a coordinated fashion so that some polypeptides are made before and others after DNA genes are packaged within the infectious particle while those needed for late transcription are present in the cytoplasm of infected cells. The aim of this project is to determine the mechanisms regulating transcription. This involves the enzymes and protein factors, the structure of the RNA and the DNA signals. Progress in understanding early transcription has been made this past year. Three components have been purified from virus particles; a multi subunit DNA-dependent RNA polymerase; a transcription initiation factor that binds specifically to early promoters and has an associated DNA-dependent ATPase; and a transcription termination factor that is a component of the capping enzyme complex. The termination signal, originally identified as TTTTINT was shown to be recognized after transcription and hence is UUUUUNU. The poly (A) polymerase from vaccinia virions was further characterized with regard to its specificity for nucleotides. The type I DNA topoisomerase from vaccinia virions was purified, the sequence of the NH2-terminus determined, the gene mapped, and the enzyme expressed in high yield in <i>E. coli</i>. The 5' ends of late mRNAs were shown to consist of capped poly (A) leaders of variable length. A transcription system that specifically transcribes late genes was developed from extracts of infected cells and a transcription factor was partially purified. The <i>in vitro</i> system synthesizes late mRNA with the 5'poly(A) leader.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT 80 characters or less Title must fit on one line between the borders

Expression of Genes of Respiratory Viruses by Vaccinia Virus Recombinants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:

B. Moss

Chief

LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20894

TOTAL MAN-YEARS:

PROFESSIONAL

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Incorporated into Z01 AI 00298-07 LVD

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00392-04 LVD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

Expression of Hepatitis B Virus Genes by Vaccinia Virus Recombinants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: B. Moss Chief LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20894

TOTAL MAN-YEARS.

PROFESSIONAL.

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither.
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Incorporated into Z01 AI 00298-07 LVD

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00393-04 LVD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (90 characters or less. Title must fit on one line between the borders.)

Expression of Malaira Genes by Vaccinia Virus Recombinants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator. (Name, title, laboratory, and institute affiliation.)

PI:

B. Moss

Chief

LVD, NIAID

COOPERATING UNITS (if any)

LABORATORY

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20894

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Incorporated into Z01 AI 00298-07 LVD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 201 AF 00416-05-LVD
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Recombinant Vaccines Against Retroviruses Associated with Leukemia and AIDS		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Others:	B. Moss P. Earl C. Flexner A. Hügin V. Karakostos S. Merli	Laboratory Chief Supervisory Microbiologist Medical Research Fellow Visiting Fellow Special Volunteer Guest Researcher LVD, NIAID LVD, NIAID LVD, NIAID LVD, NIAID LVD, NIAID LVD, NIAID
COOPERATING UNITS (if any)		
LIR, NIAID; LI, NIAID, Metabolism Branch, NCI; LTCB, NCI; Massachusetts General Hospital; Immuno AG.		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Genetic Engineering Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS 3.5	PROFESSIONAL: 2.2	OTHER: 1.3
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Human immunodeficiency virus (HIV) has been identified as the etiological agent of human immunodeficiency syndrome (AIDS). At present, there is no effective vaccine against this disease. The object of this investigation is to characterize HIV antigens, determine the targets of humoral and cell mediated immunity and to use this information to develop candidate vaccines. We have constructed recombinant vaccinia viruses containing HIV genetic information. These viruses have been used as live vaccines to immunize animals, to synthesize subunit proteins, and to make targets for cytotoxic T cells. Our finding that HIV infected individuals have circulating cytotoxic T cells directed to conserved epitopes within reverse transcriptase, as well as <i>gag</i> and <i>env</i>, may be significant for vaccine development.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Vaccinia Virus Growth Factor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R.M.L. Buller	Visiting Scientist	LVD, NIAID
Other:	B. Moss	Laboratory Chief	LVD, NIAID
	C. Duarte	Bio. Lab. Tech. (Micro)	LVD, NIAID

COOPERATING UNITS (if any)

ONCOGEN (D. Twardzik); U. Connecticut (T. Frederickson); University of Alberta, (G. McFadden)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Macromolecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

0.3

PROFESSIONAL

0.1

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|---|
| ☐ (a) Human subjects | ☐ (b) Human tissues | ☒ (c) Neither |
| ☐ (a1) Minors | | |
| ☐ (a2) Interviews | | |

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Vaccinia virus has two copies of a gene which has amino acid sequence homology with the receptor binding site of epidermal growth factor (EGF) and transforming growth factor (TGF- α). A 25 kilodalton glycoprotein (vaccinia virus growth factor [VGF] that can bind to EGF receptors) has been purified from media of virus infected BS-C-1 cells. When a mutant virus (VGF') was constructed by removing both copies of the sequence encoding the putative EGF receptor binding site, the EGF receptor binding activity in infected cells was abolished. Inoculations of the VGF mutant into [BALB/cBy] mice by the intracranial route and into the rabbit skin by the intradermal route resulted in reduced virus replication as compared to wild-type (WT) virus. No significant difference in plaqueing efficiencies between WT and VGF mutant was observed in the presence of anti-EGF receptor antibody. This and other studies support the notion that VGF binding to the EGF receptor is not required for virus entry into host cells. Experiments both *in vitro* with resting cell lines and *in vivo* with the chicken embryo CAM suggest strongly that VGF acts on uninfected cells around the focus of infection inducing cellular proliferation, which increases the rate of virus replication and spread within the lesion and, presumably, the animal. Current studies are in progress to compare the EGF- like gene products from vaccinia virus with another poxvirus, Shope fibroma virus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00445-04 LVD
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Mechanisms of Viral DNA Replication		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI: Others:	M. D. Challberg C. Wu P. D. Olivo	Senior Staff Fellow Staff Medical Staff Fellow LVD, NIAID LVD, NIAID LVD, NIAID
COOPERATING UNITS (if any) Institute of Virology, Glasgow, Scotland; Department of Microbiology, University of Michigan, Ann Arbor, MI		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Macromolecular Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20894		
TOTAL MAN-YEARS 3.6	PROFESSIONALS 2.5	OTHER: 1.35
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) Herpes simplex virus is a useful model for studying the mechanisms involved in DNA replication in eukaryotic cells. Our current efforts are directed toward studying this process with purified proteins. We have previously found seven viral genes which are both necessary and sufficient to carry out authentic origin-dependent DNA replication. The sequence of each one of these seven genes has been determined. Using the predicted amino acid sequence as a starting point, we have raised rabbit antisera against the product of each of the seven genes. The antisera have been used in immunoassay experiments to identify the proteins in HSV-infected cells. All seven proteins localize to the proteins in HSV-infected cells. All seven proteins localize to the nucleus and are expressed in a manner consistent with the idea that they are the products of early genes. Various immunological assays suggest that four of these proteins (UL5, UL8, UL9, and UL52) are made in infected cells in very low abundance relative to the other three. In UL5, UL8, UL9, and UL52 in insect cells using the baculovirus expression system. The HSV proteins made in insect cells are immunoprecipitable with the appropriate antisera and the size of each protein is indistinguishable from the corresponding protein made in HSV infected Vero cells. We have purified one of these proteins (UL9). Preliminary characterization of purified UL9 has shown that it binds specifically to the HSV origins of DNA replication.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00538-01 LVD

PERIOD COVERED

October 1, 1987 to September 30, 1988

*TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Interaction of Human Immunodeficiency Virus With the CD4 Receptor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: B. Moss
Others: E. Berger
T. Fuerst
T. Mizukami

Laboratory Chief LVD, NIAID
Expert Consultant LVD, NIAID
Guest Researcher LVD, NIAID
Guest Researcher LVD, NIAID

COOPERATING UNITS (if any)

I. Pastan and other members of the Laboratory of Molecular Biology, NCI

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS
1.7PROFESSIONAL
1.4OTHER
0.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

CD4 is an integral membrane protein of human helper T lymphocytes that serves as an essential component of the receptor for the human immunodeficiency virus (HIV), the causative agent of human immunodeficiency syndrome (AIDS). HIV binding and fusion with the cell are mediated by specific interaction between the external subunit of the viral envelope glycoprotein (gp120) and CD4 on the target cell surface. We are interested in determining the nature of the binding interaction and using this information to develop therapeutic agents. During the past year we have demonstrated that a truncated 180-amino acid fragment of CD4 contains the epitopes for several monoclonal antibodies that block HIV binding and that this CD4 fragment can form a specific complex with gp120. A recombinant fusion protein containing the CD4 binding site and portions of the Pseudomonas exotoxin A selectively killed cells infected with HIV and/or expressing the HIV envelope protein. This fusion protein has potential for AIDS chemotherapy.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Virus-cell Interactions, Viral Pathogenesis and Host Immunity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)

PI:	B. Moss	Laboratory Chief	LVD, NIAID
Other:	G. Kotwal	Visiting Associate	LVD, NIAID
	A. Hügin	Visiting Fellow	LVD, NIAID
	R. Doms	IRTA	

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

2.5

PROFESSIONAL:

1.95

OTHER:

.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Vaccinia virus contains a large number of genes that are not essential for replication in tissue culture. The conservation of these genes in vaccinia virus and other members of the orthopoxvirus genus, however, signifies that the protein products have an important role in virus-cell or virus-host interactions. The isolation of deletion mutants of vaccinia virus provides a way of identifying the "non-essential" genes. DNA corresponding to a 12,000 base pair deletion was sequenced and 17 open-reading frames were characterized. One of the open reading frames encodes a secretory protein that belongs to the family of complement binding proteins, and has a remarkable similarity to the C4b binding protein. This protein may protect vaccinia virus against host immune responses by regulating the complement cascade. Another open-reading frame, identified because the gene had been duplicated and transposed, encodes a protein that belongs to the family of plasma serum protease inhibitors. A second member of this family was located about 6,000 base pairs away. These proteins may regulate the activity of host serum proteases.

NOTICE OF INTRAMURAL RESEARCH PROJECT

201 AI-00540-01-LVD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

Molecular Biology of Human Herpesviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Niza Frenkel
Joy Steele
Eric SchirmerIPA
Medical Staff Fellow
BiologistLVD, NIAID
LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

2.0

PROFESSIONAL

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (a1) Minors☐ (a2) Interviews☒ (b) Human tissues☐ (c) Neither

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided)

Human herpesvirus 6 (HHV-6) is a new member of the herpesvirus group, first isolated in 1986 by Salahuddin et al (*Science*, 1986;234:596-601). Current data suggest that the virus is ubiquitous in humans and that infection occurs in early childhood. Our own studies of HHV-6 began in February 1988, in the new NIAID Twinbrook facility. The overall objective of our studies is to understand virus replication, and virus host-cell interactions. Our progress during the past few months can be summarized as follows:

(i) Virus propagation: A significant amount of effort in the first few months has been devoted to the methodology for virus propagation. We are now able to obtain high titer virus stocks corresponding to 10^6 - 10^9 tissue culture infectious units per ml.

(ii) Characterization of viral genome and cloning of viral DNA: We have obtained general cleavage patterns for 25 restriction enzymes. *HindIII* clones representing approximately 40% of genome were obtained thus far.

(iii) Development of genetic markers to be used in functional mapping and vector engineering: Progress has been made in selection of variants with altered cytopathology. We have also defined virus sensitivity to phosphonoacetate and to acyclovir as potentially useful markers for DNA replication enzymes.

(iv) Potential development of HHV-6 as a T cell vector: We have begun serial propagation of virus series containing defective virus genomes, so as to identify the cis-acting signals required for HHV-6 DNA propagation.

(v) Virus tropism: Preliminary results of studies done with purified T cells, B cells, and macrophages/monocytes have revealed a T cell tropism.

(vi) Interaction with the host and the requirement for virus replication: Preliminary results have revealed that HHV-6 replication requires T cell activation and that this

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI-00541-01-LVD
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Folding Assembly, and Transport of Viral Glycoproteins		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: J. Yewdell		
COOPERATING UNITS (if any) Dr. Thomas Bachi, Institute for Immunology and Virology, Zurich, Switzerland		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 5	PROFESSIONAL: 5	OTHER:
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Understanding the folding, assembly, and transport of viral glycoproteins is of general interest, and practical importance, since it could lead to greater understanding of the antigenic structure of viral glycoproteins; information critical for designing vaccines that induce antibodies that neutralize viral infectivity and confer protection against infection. We have studied the biosynthesis of influenza virus glycoproteins using monoclonal antibodies (MAbs) that discriminate between various forms of the glycoproteins present within virus infected cells. To summarize the major findings: Folding of the hemagglutinin (HA) glycoprotein occurs rapidly during biosynthesis, possibly even co-translationally. HA oligomerization begins 1 to 2 minutes following synthesis. In the presence of an agent which inhibits glycoprotein transport from the endoplasmic reticulum (e.r.), oligomerization occurs detectably within the e.r.; under normal circumstances however, oligomers are only detected in post-endoplasmic reticulum compartments. A new panel of MAbs was produced, and experiments initiated, to examine similar events in the biosynthesis and assembly of the other influenza virus glycoprotein the neuraminidase.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI-00542-01-LVD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Processing of Viral Proteins for T Cell Recognition

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. Bennink

Others: J. Yewdell

COOPERATING UNITS (if any)

LAB. BRANCH

Laboratory of Viral Diseases

SECTION

INSTITUTE AND LOCATION

NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

1.5

PROFESSIONAL

1.5

OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

T lymphocytes play a critical role in host immunity to viruses. Unlike antibodies, which recognize free antigen, T cells recognize antigens in conjunction with class I and class II molecules of the major histocompatibility complex (MHC). In previous work, we characterized the class I restricted T cell response to influenza virus at the level of individual viral gene products and class I restriction elements. In the past year, we have used this information to investigate the role of class I molecule biosynthesis and transport in cellular processing of viral antigens or T cell recognition. To summarize our findings:

1. Active ribosomal synthesis of class I molecules is not required for processing of viral antigens for T cell recognition.
2. By contrast, antigen processing is completely inhibited by incubating antigen processing cells with Brefeldin A, a fungal metabolite known to inhibit transport of secretory and membrane bound proteins at a pre-Golgi complex location.
3. Consistent with this observation, antigen processing is deficient in a mutant cell line that exhibits a defect in membrane glycoprotein transport.

LABORATORY OF MICROBIAL STRUCTURE AND FUNCTION
Rocky Mountain Laboratories
Hamilton, Montana
1988 Annual Report
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Annual Report
LABORATORY OF MICROBIAL STRUCTURE AND FUNCTION
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1987, to September 30, 1988

RESEARCH HIGHLIGHTS

Research in LMSF focuses on bacterium-host interactions. The bacterial surface components that induce or provoke disease in an animal host are studied to define mechanisms of pathogenesis and to identify possible vaccine candidates; and immunoglobulins directed against microbial surface constituents are constructed and studied to define their pathogenesis-interfering potential. Bacteria studied in LMSF during FY1988 include *Chlamydia trachomatis* and *C. psittaci*, *Francisella tularensis*, *Coxiella burnetii*, *Rickettsia rickettsii* and *R. conorii*, and *Neisseria gonorrhoeae*. Immunoglobulin reagents against group B streptococci and against HIV were explored for potential therapeutic utility.

Chlamydia trachomatis and C. psittaci: During FY1988, members of the Section of Chlamydial Diseases led by Harlan Caldwell made impressive strides toward understanding chlamydial infections and their sequelae. Earlier studies by Caldwell and coworkers showed that the dominant immunogen of *C. trachomatis* is their major outer membrane protein (MOMP), that MOMPs of different chlamydial account for their serotype distinctions, and that protection against infection correlates with mucosal immunologic responses against MOMP. Molecular cloning and sequencing of MOMP-encoding genes from several serotypes by W. Baehr, T. Joseph, Y.-X. Zhang, and Caldwell demonstrated structural similarities and differences among MOMPs of the various chlamydial serotypes. Sequence differences are concentrated in four variable domains (VDI - VDIV), all of which are surface-exposed on intact chlamydia. Those findings were extended and refined through cloning and sequencing MOMP genes from several additional chlamydial serotypes by Zhang and through sequencing the MOMP mRNAs of other serotypes by Y. Yuan. Their results pin-pointed the hydrophilic surface-exposed portions of each MOMP which constitute the molecule's major B-cell epitopes. Their data also tentatively identified two T-helper cell determinants of MOMP that likely prime animals to mount secondary IgG responses to their respective B-cell epitopes. The putative role of one such T-cell determinant has been corroborated by results with synthetic oligopeptides (N. Watkins and H. Su). A role for MOMP in the adherence of chlamydiae to host cells was defined by Su, Watkins, and Caldwell who used limited proteolysis and monoclonal antibody binding to VDII and VDI. These studies point to MOMP as a primary candidate for development of an experimental chlamydial vaccine. Accordingly, Zhang has fused selected MOMP-encoding sequences with Lamb in *Escherichia coli*, and these recombinant genes will be introduced into salmonella. Such infectious bacterial vectors should present relevant portions of MOMP to the animal host's secretory immune system in the context of gastrointestinal infection, in a fashion that is relevant for stimulating protective immunity against chlamydial infection of ocular and other mucosal sites. Specific MOMP-encoding sequences have also been inserted into the VP1 gene of Sabin 1 poliovirus by Caldwell and J. Almond (University of Reading); this recombinant vector retains infectivity for tissue culture cells and should be effective for introducing desired MOMP immunogens to an animal's mucosal and immune systems.

Previous studies by Watkins and Caldwell showed that hypersensitivity to soluble chlamydial components accounted for the reactive conjunctivitis in animals that had previous ocular infection with *C. psittaci* strain GPIC and had been rendered immune to infection with the homologous strain. R. Morrison found that such ocular hypersensitivity is directed against a 57-kDa protein that he extracted from infectious chlamydial elementary bodies and purified. This 57-kDa protein is common to both *C. psittaci* and *C. trachomatis*; it is elaborated from tissue culture cells that are chronically-infected with chlamydiae whose intracellular developmental cycle is interrupted by penicillin. The 57-kDa protein is a potent stimulator of delayed-type hypersensitivity and may be relevant to the evolution of blinding trachoma as a sequela of chlamydial infection.

Rickettsia rickettsii and conorii: Earlier studies by G. McDonald showed that a cloned gene which encodes a 155-kDa protein of *R. rickettsii* stimulates protective immunity against experimental spotted fever when administered in a whole cell recombinant *Escherichia coli* vaccine. These studies have been extended in guinea pig protection studies by McDonald. A gene of *R. conorii* (agent of Boutonneuse fever) that encodes a 197-kDa protein, immunologically related to the 155-kDa protein of *R. rickettsii*, is being cloned by S. Vishwanath. The gene encoding another *R. rickettsii* protein (120 kDa) was cloned by McDonald and N. Joste (summer student) and is being sequenced by B. Gilmore. This protein is the antigenic target for a passively-protective monoclonal antibody, has been implicated as a virulence factor for spotted fever rickettsiae, and its recombinant form will be evaluated as a potential vaccine. The roles of these proteins as determiners of rickettsial virulence will be evaluated in selected spotted fever group rickettsia that display relevant differences from *R. rickettsii*.

Coxiella burnetii: The causative agent of Q-fever, *C. burnetii*, was studied in efforts to define its propensity to incite chronic endocarditis. T. Hackstadt, S. Vishwanath, and A. Moos attempted to establish an animal model of chronic endocarditis with *C. burnetii* without success. Hackstadt and Vishwanath showed that this organism's susceptibility to killing by normal human serum correlates with structure of its lipopolysaccharide (LPS); phase I *C. burnetii* with smooth LPS were refractory to serum killing while phase II, rough LPS organisms were susceptible. Interactions of anti-protein antibodies with their antigenic targets on intact *C. burnetii* were diminished if the organisms bear smooth LPS.

Immunoglobulin biology: S. Pincus has developed an immunotoxic conjugate of ricin A chain and an anti-HIV (gp 120) monoclonal antibody in collaboration with B. Chesebro (LPVD). This immunotoxin specifically lyses tissue culture cells infected with HIV *in vitro*; it also appears to block infectivity of any HIV emanating from the target cell. Pincus and C. Horgan have initiated studies aimed at constructing immunoglobulin molecules that have desired specificities, are immunologically compatible with human use, and have enhanced Fc-mediated functions. Pincus is also exploring the optimization of monoclonal antibody structure for human use against group B streptococcal meningitis.

Francisella tularensis: F. Nano studied outer membrane proteins of *Francisella tularensis* to define their immunogenicities and to clone their respective genes. One of these, FopA, was cloned and is an immunogen that is recognized in infections by this organism.

Neisseria gonorrhoeae: Studies on gonococci concentrated on the expression and genetic control of outer membrane protein II. When predominantly protein II⁺ gonococci are intraurethally instilled into male volunteers, virtually all of the organisms shed by these experimentally-infected males were protein II⁺ (J. Swanson). Although the infecting strain of gonococci possesses a repertoire of at least nine different protein II species, one particular protein II⁺ variant (P.IIc⁺) dominated early in the urethral infections of three men. Five different protein II species were produced by gonococci later in one man's infectious course. Eight different genes that encode protein II species in a single strain were cloned and several were sequenced by P. van der Ley. Three distinct genes encode an identical protein II in this strain; but this particular protein II is expressed no more frequently than others that are encoded by a single chromosomal gene. Expression of each protein II species is switched when its OFF, "out of frame" gene undergoes apparent gain or loss of nucleotide pentamers (CTTCT) to effect a frameshift-mutation; the transcription initiation site and the mature protein II-encoding sequences are brought "in frame" and the gene is ON. This novel mechanism for genetic control is being explored by B. Belland who inserted TnPhoA elements into recombinant protein II genes so that ON-OFF switching could be easily visualized. The apparent addition and deletion mutations that switch protein II genes ON-OFF are independent of recombination in both *E. coli* and *N. gonorrhoeae*. Belland and van der Ley demonstrated that a recombinant-encoded protein II is exposed on *E. coli* much the same as in *N. gonorrhoeae*.

ADMINISTRATIVE REPORT

Personnel changes during the past year include arrival of four Senior Staff Fellows (Rick Morrison, LPVD, RML; Kolari Bhat, Univ. Pennsylvania; Paul Policastro, Scripps Institute; Patty Rosa, Medical Biology Inst. La Jolla), three IRTA Fellows (Stuart Hill from Univ. Montana, Bob Gilmore and Scott Manning, both from Oregon State Univ.), and one Visiting Fellow (Yuan Ying, Beijing Inst. of Ophthalmology). Leaving LMSF were Senior Staff Fellows Fran Nano (to Univ. Victoria, B.C.), Ted Hackstadt (to Univ. Texas Med. Center, Galveston), Wolfgang Baehr (to Baylor Univ.), Visiting Fellow Peter van der Ley (to Nat'l. Inst. Public Health & Env. Hygiene, Bilthoven, The Netherlands), Staff Fellow Abbie Moos, and Guest Researcher Theresa Joseph. Changes in technical staff including departure of Ken Robbins and Theresa Brown (to CDC) and the arrival of Sandra Morrison. Summer students in LMSF included Michael Jasemback, Mike Johnson, and Kathy Wells from Carroll College and Nancy Joste from Univ. New Mexico College of Medicine. Seminars were given by a number of visiting scientists, including the following: J.W. Almond (Univ. Reading, U.K.), David Stewart (CSIRO, Australia), Tom Silhavy (Princeton), and Martin Lavin (Univ. Queensland, Australia). In addition, a number of potential LMSF staff members visited RML and delivered seminars: W. Barry (ILRAD, Kenya), J. Rader (No. Carolina St. Univ.), D. Portnoy (Washington U.), R. Monk (Alton Jones Cell Center), J. Kiehlbauch (Univ. Washington), C. Roessner (Texas A&M), K. Takeuchi (Roswell Park), R. Rubin (Biotechnica Inc.), D. Glick (Seattle Biomed. Res. Inst.), and C. Elkins (Hahnemann Univ.).

HONORS AND AWARDS

Journal Editorial Boards:

J. Swanson - Infection and Immunity

Manuscripts were reviewed by LMSF staff for the following journals: Canadian Journal of Microbiology, Cell, Infection and Immunity, Journal of Bacteriology, Journal of Biological Chemistry, Journal of Clinical Investigation, Journal of Clinical Microbiology, Journal of General Microbiology, Journal of Immunology, Journal of Infectious Diseases, Journal of Rheumatology, Microbial Pathogenesis, Proceedings of the National Academy of Sciences USA, Science, and Sexually Transmitted Diseases.

Professional Posts:

O. Barrera - Promotion Advisory Committee, NIAID

H. Caldwell - Ad Hoc Member, Microbiology and Infectious Diseases Research Committee, NIAID
Faculty Affiliate, Department of Microbiology, University of Montana,
Missoula, MT

Invited Lectures and Participation in Meetings and Symposia:

J. Swanson - STD retreat, University of California, San Francisco, CA
Thomas Alva Edison Foundation's 1988 Montana Science Institute, "Science and Technology: Road Map to our Future," Bozeman, MT
Third Shipley Symposium, Harvard Medical School, Boston, MA

H. Caldwell - American Society for Microbiology, Miami, FL
Federation of American Societies for Experimental Biology, Las Vegas, NV

T. Joseph - Federation of American Societies for Experimental Biology, Las Vegas, NV

G. McDonald - University of Tennessee, Memphis, TN
Session moderator and participant, American Society for Rickettsiology and Rickettsial Diseases, Santa Fe, NM

R. Morrison - Federation of American Societies for Experimental Biology, Las Vegas, NV

F. Nano - University of Victoria, Victoria, BC, CANADA
University of Alberta, Edmonton, Alberta, CANADA
University of Idaho, Moscow, ID
Boehringer-Mannheim Biochemicals, Indianapolis, IN
Medical College of Ohio, Toledo, OH
University of Wisconsin, Madison, WI

S. Pincus - Arthritis Foundation Scientific Meeting, Jacksonville, FL

H. Su - American Society for Microbiology, Miami, FL

N. Watkins - Federation of American Societies for Experimental Biology, Las Vegas, NV

Y. Zhang - American Society for Microbiology, Miami, FL

Other Activities:

- H. Caldwell - Reviewed research grants for Medical Research Council, Canada, National Science Foundation, Washington, DC, and The Edna McConnell Clark Foundation, New York, NY
- S. Pincus - Reviewed research grants for Arthritis Foundation.
- J. Swanson - Reviewed research grants for National Science Foundation, Washington, DC

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00193-09 LMSF

PERIOD COVERED

October 1, 1987, to September 30, 1988

TITLE OF PROJECT: 80 characters or less. Title must fit on one line between the borders.

Gonococcal Surface Components: Structure and Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)

PI:	J. Swanson	Chief	LMSF, NIAID
Others:	P. van der Ley	Visiting Fellow	LMSF, NIAID
	R. Belland	Visiting Fellow	LMSF, NIAID
	S. Hill	IRTA Fellow	LMSF, NIAID
	O. Barrera	Microbiologist	LMSF, NIAID
	K. Robbins	Microbiologist	LMSF, NIAID
	D. Corwin	Bio. Lab. Tech.	LPB, NIAID
	S. Morrison	Microbiologist	LMSF, NIAID

COOPERATING UNITS (if any)

John Boslego, WRAIR, Washington, D.C.
Michael Koomey, Rockefeller University

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

6.05

PROFESSIONAL

3.2

OTHER:

2.85

CHECK APPROPRIATE BOXES:

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The surface components of gonococci (Gc) dictate this bacterium's pathogenic personality. Pili are thought to initiate and maintain adherence of Gc to a host mucosae, thereby promoting colonization and infection. This is correlated with virulence of pilus⁺ and avirulence of pilus⁻ Gc in human volunteers. Previous work from this laboratory focused on structural variation among pili elaborated by Gc recovered from experimental human infections, both in terms of their antigenic diversity and the mechanisms responsible for such changes. Current work is directed toward illuminating similar questions about another set of Gc surface components—the outer membrane protein II (P.II) family. When expressed, each P.II is a major component of the outer membrane, but production of these polypeptides has no known consequences for Gc cultivated *in vitro*. Experiments during the past year have concentrated on expression of Gc recovered from males that were experimentally infected with P.II⁺ organisms. These input P.II⁺ Gc were quickly replaced by P.II⁺ variants, and from this strain's repertoire of 12+ P.II species, P.IIc was dominant among reisolated organisms. In addition to this survey of P.II expression by infecting Gc, studies were conducted on the genetic control of these outer membrane polypeptides. Several P.II genes were cloned from a single strain and were then sequenced. *In vitro* mutagenesis was used to determine the mechanism by which P.II genes that are "out-of-frame" display low level expression in *E. coli*. Cloned P.II genes into which TnphoA has been inserted as reporter molecules were constructed to examine the processing and transport of protein II to the Gc outer membrane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00216-08 LMSF
PERIOD COVERED October 1, 1987, to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunology of Chlamydial Surface Antigens		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	H. D. Caldwell	Research Microbiologist LMSF, NIAID
Others:	Y.-X. Zhang	Visiting Associate LMSF, NIAID
	H. Su	Visiting Fellow LMSF, NIAID
	Y. Yuan	Visiting Fellow LMSF, NIAID
	W. B. Baehr	Senior Staff Fellow LMSF, NIAID
	S. Manning	IRTA LMSF, NIAID
	S. Stewart	Microbiologist LMSF, NIAID
COOPERATING UNITS (if any) Theresa Joseph, Foundation for Advanced Education in Science; Jeffrey Almond, Department of Microbiology and Immunology, University of Reading, Reading, U.K.; and Hugh Taylor, Johns Hopkins University School of Medicine, Baltimore, MD		
LAB/BRANCH Laboratory of Microbial Structure and Function, Hamilton, MT 59840		
SECTION Chlamydial Diseases Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.55	4.05	2.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The immediate goals of this project are to define and molecularly characterize chlamydial surface components that are virulence determinants or antigens of importance in the hosts immune response to chlamydial infection. The long-term objectives are to: (i) understand how chlamydiae interact with and infect eukaryotic cells, (ii) define pathogenetic mechanisms of chlamydial disease, (iii) identify target antigens for development of a recombinant vaccine to prevent and/or control chlamydial diseases in humans. We have previously shown that the immune response to chlamydial infection has both protective and deleterious components. The antigens associated with these responses are distinct. The protective antigen is the major outer membrane protein (MOMP) and the deleterious antigen is a 57-kDa protein. The MOMP genes from several <i>C. trachomatis</i> serovars have been cloned and sequenced. Serotype, serogroup, and species-specific epitopes have been located on MOMP with a panel of protective monoclonal antibodies (MAbs). Sequence analysis showed the MOMP genes encode proteins with highly conserved regions interrupted symmetrically by four short variable domains (VD). The VDs possess contiguous antigenic determinants recognized by protective MAbs. Surface proteolysis of intact chlamydiae with trypsin selectively cleaves within these VDs and abolishes attachment of chlamydiae to HeLa cells. These findings implicate MOMP as a chlamydial adhesion and suggest that the contiguous sequences within these VDs are potential vaccine candidate antigens. Current studies are directed at identification of MOMP T-cell determinants and the construction of live enteric vaccine vectors expressing co-linear protective MOMP B-cell determinants and T-helper cell determinants as antigenic hybrid proteins. Protective MOMP sequences are being cloned and expressed as antigenic hybrids of the LamB protein in <i>S. typhimurium</i> and VP1 of the Sabin type 1 vaccine strain. The immunogenicity and vaccine efficacy of these infectious recombinant vectors is currently being evaluated.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00234-C6 LMSF
PERIOD COVERED October 1, 1987, to December 31, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Biology of Intracellular Parasitism		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator) (Name, title, laboratory and institute affiliation)		
PI:	T. Hackstadt	Senior Staff Fellow LMSF, NIAID
Others:	S. Vishwanath	Visiting Associate LMSF, NIAID
COOPERATING UNITS (if any)		
LAB BRANCH Laboratory of Microbial Structure and Function, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS	PROFESSIONAL	OTHER:
6	35	25
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) The pathogenesis of <i>Coxiella burnetii</i> infections was studied in rabbits and in vitro. We studied (a) the pathogenesis of experimental endocarditis, (b) the effect of human serum complement on lipopolysaccharide (LPS) phase variants and (c) the role of LPS in phase variation. A. <u>Endocarditis.</u> <i>C. burnetii</i> strains from acute Q fever and from endocarditis patients differ in LPS structure and in their ability to induce fever in animals. Studies using a rabbit model of endocarditis showed no differences between the abilities of the Nine Mile strain (typical of acute Q fever strains) and the Priscilla strain (typical of endocarditis strains) to colonize catheter-induced cardiac vegetations. An in vitro assay showed no differences between these strains in their abilities to adhere to fibrin-platelet clots. B. <u>Serum complement-susceptibility.</u> The rough LPS phase variant was serum sensitive, whereas the smooth and intermediate LPS phase variants were resistant. The smooth and intermediate phase variants, however, differed in their interactions with the complement system, suggesting that the mechanisms by which they resisted complement-killing were different. C. <u>Role of LPS in phase variation.</u> The smooth LPS on native phase I variants sterically blocked the binding of antibodies to surface proteins shared with phase II cells. Although any of these surface-exposed proteins which are accessible to antibodies on phase II cells may therefore confer apparent phase II serospecificity, these studies suggest that the unique phase determinant is in the phase II LPS. The significance of this project lies in defining host defence mechanisms controlling <i>C. burnetii</i> infections, in determining whether certain strains have a predilection to cause endocarditis, and in developing effective antimicrobial therapy for <i>C. burnetii</i> endocarditis.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00413-04 LMSF

PERIOD COVERED

TITLE OF PROJECT (90 characters or less. Title must fit on one line between the borders.)

Molecular Genetics of *Chlamydia trachomatis*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: F. E. Nano

Senior Staff Fellow

LMSF, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrebated type. Do not exceed the space provided.)

This project was terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00441-04 LMSF

PERIOD COVERED

October 1, 1987, to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Rickettsia rickettsii Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: G. A. McDonald Staff Fellow LMSF, NIAID

Others: R. D. Gilmore IRTA LMSF, NIAID
S. Vishwanath Visiting Associate LMSF, NIAID
R. E. Mann Bio. Lab. Tech. LMSF, NIAID

COOPERATING UNITS (if any)

None

LAB. BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

3.9

PROFESSIONAL

2.9

OTHER:

1.0

CHECK APPROPRIATE BOXES:

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are interested in the characterization of two proteins of *Rickettsia rickettsii*, the etiological agent of Rocky Mountain spotted fever (RMSF). These two proteins (120 and 155 kDa) are immunogenic, have been implicated as virulence factors, and may be subunit vaccine candidates. We cloned the gene encoding the 155-kDa protein and showed that immunization with *Escherichia coli* producing this protein protected guinea pigs challenged with viable *R. rickettsii* 7 days postvaccination. These studies were extended to determine whether boosting the animals 7 days after the first immunization would enhance the protection. The gene encoding a 197-kDa protein in *R. conorii*, the cause of Boutonneuse fever, is also being cloned; this protein is of interest because it is immunologically related to the 155-kDa protein of *R. rickettsii*. We also cloned the *R. rickettsii* gene encoding the 120-kDa protein and successfully subcloned a portion of the gene into bacteriophage lambda-gt11. *E. coli* strain Y1090, made lysogenic for the recombinant bacteriophage (lambda-NEJ120), produced a 130-kDa protein that reacted by immunoblotting with a monospecific polyclonal serum raised to the 120-kDa protein; this protein was shown to be a fusion product of the 120-kDa rickettsial protein and β -galactosidase. The 3.7-kilobase (kb) insert of lambda-NEJ120 was subcloned into the plasmid vector pUC19 to yield pGAM120, portions of which were subcloned into M13 vectors for DNA sequencing. The pGAM120 insert was used as a probe to screen a *R. rickettsii* (R strain) genomic library made in lambda-EMBL3 and a *R. rickettsii* (HLP strain) library made in pBR322; the HLP strain is avirulent in guinea pigs and is also thought to be avirulent in humans. Positive recombinants were obtained from both libraries. We have also continued our efforts to evaluate the use of monoclonal antibodies to therapeutically treat RMSF.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00442-04 LMSF
PERIOD COVERED October 1, 1987, to October 31, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunobiology of Guinea Pig Inclusion Conjunctivitis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: N. G. Watkins Senior Staff Fellow LMSF, NIAID Others: A. B. Moos Staff Fellow LMSF, NIAID		
COOPERATING UNITS (if any) Richard Morrison, Foundation for Advanced Education in Science		
LAB/BRANCH Laboratory of Microbial Structure and Function, Hamilton, MT 59840		
SECTION Chlamydial Diseases Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 0.2	PROFESSIONAL: 0.2	OTHER:
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Corneal opacity and vascularization occurs following repeated or chronic chlamydial ocular infections and may result in blindness. The mechanisms resulting in corneal opacity are unknown but may be caused by hypersensitivity to chlamydial components. To study corneal involvement during chronic chlamydial infections, we have developed an animal model using guinea pigs treated topically with penicillin during infection with <i>C. psittaci</i> strain GPIC. Corneal opacity and vascularization occurs only in animals simultaneously infected with GPIC agent and treated with penicillin. Opacity resolves with clearance of GPIC agent from the conjunctivae; however, the cornea sustains permanent damage as evidenced by vascularization and scarring observed months after resolution of opacity. This animal model will be useful to study the role of chlamydiae and chlamydial components in the development of corneal damage during chlamydial infections. <i>C. psittaci</i> strain GPIC can infect the genital tract in addition to the conjunctiva. We have utilized this model to determine if hypersensitivity to chlamydiae occurs in the oviduct and uterus of guinea pigs recovered from a previous vaginal infection. The uteri and oviducts of these animals develop significant inflammation following inoculation of an extract of <i>C. psittaci</i> strain GPIC. This inflammation needs to be further characterized to determine whether it is characteristic of delayed hypersensitivity or of cellular toxicity.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00515-01 LMSF

PERIOD COVERED

October 1, 1987, to July 5, 1988

TITLE OF PROJECT (50 characters or less. Title must fit on one line between the borders.)

Molecular Analysis of *Francisella* Surface Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator.) (Name, title, laboratory and institute affiliation)

PI: F. E. Nano

Senior Staff Fellow

LMSF, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

.75

PROFESSIONAL

.75

OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

During the past year, I took the first steps in developing *Francisella tularensis* into a useful model of bacterial pathogenesis. I attempted to introduce exogenous DNA into *Francisella* using a variety of methods, and succeeded by transforming recombinant clones containing transposon insertions. The recombinant clones apparently integrated into the chromosome, thus preparing the way for gene replacement studies. I have also cloned the genes encoding five presumptive outer membrane proteins. One of the clones expresses a heat and 2-mercaptoethanol modifiable protein. This protein, FopA, is one of the major immunogens seen in *Francisella* infections.

Dr. Nano has moved, therefore this project is terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00516-01 LMSF
PERIOD COVERED October 1, 1987, to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunoglobulin Biology		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	S. H. Pincus	Expert LMSF, NIAID
Others:	C. J. Horgan	Senior Staff Fellow LMSF, NIAID
	B. W. Chesebro	Chief, LPVD LPVD, NIAID
	R. L. Cole	Chemist LMSF, NIAID
	K. Wehrly	Chemist LPVD, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Microbial Structure and Function, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.75	2.0	75
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The goal of this work is to understand the structure and function of the immunoglobulin molecule. The work is being performed so that this understanding will be applied to the development of antibodies for human therapy. A. Therapeutic Systems. 1. Antibodies to surface antigens of group-B streptococci have been demonstrated to have protective efficacy in a model of neonatal sepsis. We are now examining the pharmacologic characteristics of these antibodies in neonatal animals including: body distribution, long-term persistence, dose and appropriate mixtures of different monoclonals to protect against all serotypes of group-B streptococci. 2. The <i>in vitro</i> efficacy of anti-HIV envelope (gp 120) monoclonal antibodies has been tested. Combinations of antibody, complement, and leukocyte populations are only marginally effective in inducing lysis of HIV-infected targets. Antibody coupled to ricin A-chain is highly lethal to infected targets while sparing uninfected cells. This immunotoxin also blocks infectivity of the targeted cell. We are beginning <i>in vivo</i> studies to test the efficacy of antibody based therapies of AIDS. B. Genetically Engineered Antibodies. Vectors have been prepared carrying human mu, gamma-1, and gamma-4 constant region genes in an enhancer containing expression vector. Variable region genes from anti-(T,G)-A--L antibodies have been inserted. DNA has been transfected into cell lines by electroporation and expressed as functional antibody. Variant hinge region genes are being constructed. These will be used to study the role of the antibody hinge in Fc-mediated effector function. C. Idiotype. The idiotypic network induced in C57BL/10 mice by immunization with the synthetic polypeptide (Tyr, Glu)-Ala--Lys is being explored. Monoclonal antibodies representing antibody 1 (ab 1), ab 2, and ab 3 have been made and sequences obtained. Transgenic mice that over-express the ab 1 heavy chain have been bred. The effect of this over-expression on the network is being studied.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 AI 00518-01 LMSF

PERIOD COVERED

October 1, 1987, to September 30, 1988

TITLE OF PROJECT: 80 characters or less. Title must fit on one line between the borders.

Antigenic Mapping of Chlamydial Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator): Name, title, laboratory, and institute affiliation

PI: N. G. Watkins Senior Staff Fellow LMSF, NIAID
Others: H. D. Caldwell Research Microbiologist LMSF, NIAID
Su Hua Visiting Fellow LMSF, NIAID

COOPERATING UNITS (if any)

Theresa Joseph, Foundation for Advanced Education in Science

LABORATORY

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

Chlamydial Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

1.1

PROFESSIONAL

1.1

OTHER

0

CHECK APPROPRIATE BOXES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The major outer membrane protein (MOMP) of *Chlamydia trachomatis* has been identified as a possible immunogen for vaccine development. The objectives of this project are to identify amino acid sequences of MOMP which are surface accessible to immunoglobulins and to determine if these sequences are immunodominant during infection. Peptides containing sequences of the four variable domains (VDI, VDII, VDIII, and VDIV) have been synthesized. These peptides have been used to define the epitope binding site of MAbs to MOMP, in particular, of MAbs which are known to passively protect against infection in the monkey eye or in the mouse toxicity test. The antibody response during infection to the VDs of *C. psittaci* strain GPIC will be tested using synthetic peptides of all four VDs of GPIC and testing sera and tears from infected and immune guinea pigs. Polyclonal rabbit antisera is being raised to VDIII of A, E, and L2 MOMP to determine if this VD is surface accessible and to examine the homology of VDIII among serovars. Trypsin cleavage of MOMP at the surface of viable EBs results in loss of binding of EBs to HeLa 229 cells. Loss of binding occurs only when B MOMP is cleaved in both VDII and VDIV, suggesting a role for MOMP in interactions of chlamydiae with host cells. Synthetic peptides corresponding to VDII and VDIV of B and L2 serovars have been synthesized and will be used to determine their role in binding of chlamydiae to HeLa 229 cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00519-01 LMSF
PERIOD COVERED January 31, 1988, to September 30, 1988		
TITLE OF PROJECT (30 characters or less. Title must fit on one line between the borders.) Chlamydial Antigens: Role in Pathogenesis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	R. P. Morrison	Senior Staff Fellow LMSF, NIAID
Others:	H. D. Caldwell N. G. Watkins	Research Microbiologist LMSF, NIAID Senior Staff Fellow LMSF, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Microbial Structure and Function, Hamilton, MT 59840		
SECTION Chlamydial Diseases Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS.	PROFESSIONAL:	OTHER:
0.9	0.9	0
CHECK APPROPRIATE BOXES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (at) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Ocular infection of guinea pigs with <i>C. psittaci</i> strain GPIC causes a self-limiting conjunctivitis similar to that observed during primary ocular infections of humans infected with <i>C. trachomatis</i>. We have used a guinea pig model to study the immunological responses elicited by an ocular chlamydial infection. The transient protective immunity that develops after primary infection is thought to be mediated by local antibody. We have begun to analyze tears and serum for antibodies directed at particular chlamydial antigens. This may allow us to correlate antibody response to a particular antigen and protection. Preliminary data indicate that immune sera contain antibodies reactive to many chlamydial antigens, whereas antibodies found in immune tears react primarily with proteins having molecular weights of 92 kDa, 84 kDa, 57 kDa, and MOMP. IgG₂ was found to be the predominant isotype of chlamydial-specific serum antibodies, whereas tears contained approximately equal titers of IgA and IgG₂. An important sequelae of repeated or recurrent chlamydial infections is chronic inflammation, which leads to irreversible tissue damage. We have identified and purified a 57-kDa genus-specific chlamydial antigen that elicits an ocular delayed hypersensitivity response in immune guinea pigs. Furthermore, when cells persistently infected with <i>Chlamydia</i> are maintained <i>in vitro</i>, this antigen is released into the medium. The delayed hypersensitivity response elicited by antigen purified from culture supernatants of persistently infected cells is indistinguishable from that elicited by antigen purified from chlamydial EBs. The 57-kDa chlamydial protein is a potent stimulator of ocular delayed hypersensitivity and may contribute to the chronic inflammation that is observed in human chlamydial disease.		

LABORATORY OF PATHOBIOLOGY
Rocky Mountain Laboratories
Hamilton, Montana
1988 Annual Report
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ANNUAL REPORT
LABORATORY OF PATHOBIOLOGY
ROCKY MOUNTAIN LABORATORIES
HAMILTON, MONTANA
NATIONAL INSTITUTES OF ALLERGY AND INFECTIOUS DISEASES
OCTOBER 1, 1987, TO SEPTEMBER 30, 1988

Members of the Laboratory of Pathobiology bring a broad range of skills and experience to bear in their research. Modern techniques of molecular biology are used in several medically important systems to characterize critical aspects of host-pathogen interaction. Significant progress has been made this past year in first defining and then exploiting features of these systems which might result in improved diagnosis and/or treatment. Basic information on pathogenesis is accumulating as well. The broad interaction among members of the laboratory coupled with a strong guest researcher program insures that the best experimental approaches are incorporated effectively into the programs of the laboratory. The Laboratory of Pathobiology functions in four major experimental groups emphasizing two human pathogens - Bordetella pertussis and Borrelia burgdorferi. Dr. Claude Garon serves as Laboratory Chief.

The Molecular Pathobiology Section under the direction of Dr. Jerry Keith has continued to focus on the molecular cloning and expression of genes involved in the production of the toxic components of Bordetella pertussis. The S1 subunit of pertussis toxin is an enzymatically active protein that catalyzes the transfer of ADP-ribose from NAD to guanine nucleotide regulatory proteins in eukaryotic cell membranes. The gene encoding the S1 subunit, as well as several truncated versions, has been expressed in Escherichia coli using vectors containing the transcriptional and translational control elements of the lac operon. By employing these recombinant S1 subunit constructs, the structure and function relationship between pertussis toxin, pathogenesis, and immunoprotection have been assessed. Using the results of these studies as a basis for further research, a region of the S1 subunit has been identified that is required for enzymatic activity and that contributes to the formation of a neutralizing antigenic determinant. The gene encoding the S1 subunit was modified in this critical region by site-specific mutagenesis. One of the mutants in which a single amino acid substitution was engineered into the S1 subunit had no enzymatic activity but retains the protective epitope. The genetic changes responsible for the loss of enzymatic activity are now being engineered into the chromosome of B. pertussis to develop a new strain of the bacteria which will produce a genetically detoxified pertussis toxin. This modified toxin has important potential for use as a component of an improved acellular vaccine against whooping cough. In initial experiments, immunization with recombinant pertussis toxin subunits S1 and S2 produced in E. coli did not yield protection which was greater than that provided by the vehicle alone. However, following inclusion of active toxin in the protocol, immunoprotection by S1 and S2 reached 70% of the level provided by the whole cell vaccine which served as a positive control. Deletion and substitution mutants of the S1 subunit are currently being tested to identify the epitopes associated with protection from lethal challenge. In addition, S1 based peptides are being synthesized to test their protective potential.

Lyme disease is now the most common arthropod-borne disease in the United States. While originally thought to be confined to limited areas, the disease has now been found in at least 32 states and 6 continents. Members of the laboratory have made a major commitment to an improvement in diagnostic reagents and to an understanding (in molecular terms) of how this pathogen produces its long list of clinical features. The Arthropod-borne Diseases Section under the direction of Dr. Tom Schwan has concentrated on this and other vector-borne pathogens. Recently this section has demonstrated that continuous cultivation of the Lyme disease spirochete in modified Kelly's medium causes a reduction in the number of detectable plasmids and the loss of infectivity in the white-footed mouse. In an unpassaged culture of Borrelia burgdorferi, nine plasmids were present, including seven linear plasmids ranging in size from 49 to 16 kilobases (kb) and two circular plasmids of 27 and 7.6 kb. The 7.6 kb circular and 22 kb linear plasmids were no longer detectable in spirochetes noninfective in white-footed mice, suggesting that gene(s) encoding for factors responsible for infection may be present on one or more of these extrachromosomal elements. Furthermore, changes in spirochetal proteins and lipopolysaccharide-like material were observed also during early cultivation and may be related to loss of infectivity. The 7.6 kb circular plasmid present in several low passage strains of B. burgdorferi has been identified as a strong candidate for both regulating infection and as a marker for potentially infectious strains. This plasmid has been purified and restriction fragments of approximately 200 and 500 base pairs have been cloned, and are being tested as DNA hybridization probes.

Also participating in research on the molecular biology of the Lyme disease spirochete is the Pathobiology Section under the direction of Dr. Claude Garon. Earlier descriptions of covalently closed, double-stranded, linear plasmids in Borrelia burgdorferi by members of this laboratory has led to a more careful analysis of several low passage strains by agarose gel electrophoresis and by nucleic acid electron microscopy. The plasmid pool of one isolate contained nearly 200 kb of unique DNA sequences including both linear (5.4, 19.5, 21.5, 31, 36, and 53 kb) and circular (26 kb) molecules. Since accurate size determination by agarose gel electrophoresis is not possible with an undefined mixture of linear and circular plasmids, a new method was developed to identify molecular form in these samples without band purification or electron microscopy. While all of the linear species rapidly reannealed to linear duplexes after alkaline denaturation, single-stranded circular molecules measuring twice the length of the linears could be produced by treatment with methyl mercury, glyoxal and urea prior to mounting for microscopy. Both circle formation and rapid reannealing could be prevented by pre-treatment with single-strand specific nucleases. Molecules could then be compared by heteroduplex analysis. One of the linear plasmids, in addition to its covalently closed structure, appeared to possess a unique inverted redundancy of 2.3 kb located near the molecular termini. Two prominent stem-loop structures possibly suggesting transposable elements were detected as well. Electroporation experiments have demonstrated the feasibility of developing a genetic transfer system in B. burgdorferi. Studies on the replication of these linear plasmids, with their terminal hairpin structures (which may resemble telomeres of eukaryotic chromosomes) are underway as well.

The Immunopathology Section, with Dr. John Munoz as Acting Head, has continued during this past year to characterize the immunopotentiating action of small quantities of crystalline pertussis toxin (Ptx). These studies have acquired added importance given the role of Ptx in boosting protective antibody response. Ptx increased strikingly the protective action of vaccines made from Bordetella pertussis strains lacking Ptx, or from strains in which a genetically modified gene for the S1 subunit of Ptx was present. Ptx also enhanced the protective activity of vaccines made from B. parapertussis and B. bronchiseptica, but had little effect on vaccines made from wild-type strains of B. pertussis known to produce Ptx. The antibody response to surface antigens of Bordetella was also studied. It was shown that the antibodies of the IgG class are increased with all the vaccines used, the IgM is only slightly increased, and the IgA class is not increased. However, the IgE class of antibodies was noticeably increased in vaccines containing Ptx or when Ptx was included in the immunization with vaccines lacking Ptx. It was previously shown that any modification of the gene responsible for the production of the S1 subunit of Ptx, made the B. pertussis cells ineffective in all the immunopotentiating actions found in vaccines made from wild-type strains containing the intact toxin gene. These observations have been extended to vaccines made with B. parapertussis into which unmodified Ptx genes, or genes modified in the S1 region or other subunits had been introduced. Vaccines made from B. parapertussis with the intact gene had all the immunopotentiating activities of the wild-type B. pertussis, including the induction of protection against intracerebral (IC) challenge. B. parapertussis mutants containing a modified S1 gene or a gene lacking S1 did not have any of the immunomodulating activities of wild-type B. pertussis cells. The activities measured were: histamine sensitization, leukocytosis promotion, enhancement of anaphylaxis, enhancement of IgE production, increase in the percentage of IgE containing cells in the spleen and the ability to protect mice from IC challenge with virulent B. pertussis. Vaccines made from B. pertussis strains that do not have an intact Ptx gene, when given alone, stimulate only weak protection of mice against B. pertussis. However, when in addition 20-100 ng of Ptx are given, these vaccines become as efficient in protecting mice as vaccines made from wild-type B. pertussis. Vaccines made from B. parapertussis strains are only weakly protective against IC challenge with B. pertussis, however, strains into which the Ptx gene had been introduced became highly effective in inducing protection. As in the case of B. pertussis strains, the B. parapertussis strains containing a Ptx gene in which the S1 gene had been modified were not any more protective than the carrier strain of B. parapertussis. If, in addition to these weakly protective vaccines, Ptx was given, the vaccines became highly effective.

ADMINISTRATIVE

Dr. Claude Garon was appointed Chief of the Laboratory of Pathobiology during the year. Dr. Willy Burgdorfer, Scientist Emeritus, has continued to provide valuable support for the Arthropod-borne Diseases Section.

Guest Researchers in the Laboratory of Pathobiology have included: Dr. Stanley Falkow (Stanford University School of Medicine), and Kirsten Vadheim (Montana State University). Joining the laboratory were: Dr. Stephanie Czub (Free University of Berlin) as a Visiting Fellow; Dr. Warren Simpson (University of Minnesota) as a Visiting Associate; and Dr. David Dorward (University of Montana) as a Staff Fellow.

Visitors/Collaborators who spent varying amounts of time in the laboratory during the past year included: Dr. Tracy Heimberger (Richmond, VA); Dr. Mark Peppler (Calgary University, Calgary, Alberta, Canada); Dr. Robert Quackenbush (NIH); Dr. Vince Marshall (Council Bluff, IA); Dr. Andrea Dlesk (Marshfield Clinic, WI); Dr. J. E. Ricketts (Huntington, WV); Dr. Durland Fish (New York Medical College, Valhalla, NY); Dr. Marie Bloch (Merieux, Lyon, France); Dr. Brenda Spangler (Argonne National Laboratories, Argonne, IL); Dr. Paul Lavoie (San Francisco, CA); Dr. Pierre Lallouette (Paris, France and Salk Institute); Dr. Tom Quan (Centers for Disease Control, Fort Collins, CO); Dr. Lucy Tompkins (Stanford University, San Francisco, CA); Dr. Al Jesaitis (Scripps Institute, San Diego, CA); Dr. Melanie Spriggs (LID, NIH); Dr. Martin Rodbell (NIEHS, NIH); Dr. Drucilla Burns (FDA); Drs. Hiroko and Yuji Sato, NIH, Tokyo, Japan); and Dr. Alex Bonner (Milligen, Bedford, MA).

The Laboratory of Pathobiology has undertaken to organize an International Workshop at Rocky Mountain Laboratories to bring together a panel of the most knowledgeable workers to explore in detail the latest concepts on the pathobiology of Bordetella pertussis. The meeting which featured over 100 scientists received both institute and industry support and was held at the Hamilton facility in August 1988.

HONORS AND AWARDS

Dr. Tom Schwan

Invited speaker:

Department of Microbiology, University of Minnesota, October 1987
Society of Vector Ecology, Asilomar, California, November 1987
Department of Microbiology, University of Alabama, April 1988
Department of Microbiology, University of Montana, May 1988
U.S. Forest Service, West Fork, Montana, May 1988
American Ornithologists Union, Fayetteville, Arkansas, August 1988

Reviewed manuscripts for:

Journal of Medical Entomology
Journal of Wildlife Diseases
Journal of Clinical Microbiology
Clinical Microbiology Reviews

Dr. John Munoz

Reappointed as a staff affiliate of the University of Montana.

Trustee of the Stella Duncan Memorial Research Fund, University of Montana.

Reviewed manuscripts for:

Infection and Immunity
Immunopharmacology
Clinical Immunology and Immunopathology

Helped organize the International Workshop on Bordetella pertussis held at Rocky Mountain Laboratories, August 18-20, 1988.

Attended and presented a paper at the FEMS Symposium on Pertussis, East Berlin, Germany, April 20-22, 1988.

Invited to become a chapter member of "The International Endotoxin Society."

Dr. Claude Garon

Received NIH Director's Award - June 6, 1988

Invited to serve on State Board of Governors for Montana Biotechnology Foundation.

Invited to present a lecture at the Seventh Pfefferkorn Conference on the Science of Biological Specimen Preparation for Microscopy and Microanalysis, London, England.

Invited contributor to the Public Broadcasting Service's science series called "The Infinite Voyage."

Invited lecturer at National Medical Library Association Annual Meeting, New Orleans, LA.

Invited speaker:

Westview Junior High School, Hamilton.

Continuing Education Series for Science Teachers on Genetic Engineering, Missoula.

University of Montana - Department of Microbiology, Missoula.

International Students Association - University of Montana, Missoula.

Molecular Biology Seminar Series - University of Montana - Department of Chemistry.

Hamilton Lions Club

Dr. Jerry Keith

Attended the IACCA Meeting October 4-7, 1987, in New York City, NY.

Invited speaker at Notre Dame, NY, January 17-19, 1988.

Invited speaker at NIAID Pertussis Vaccine Meeting, Bethesda, MD, February 8-9, 1988.

Invited speaker, Smith Kline/Rit, Brussels, Belgium, April 18, 1988.

Invited speaker at the FEMS Pertussis Symposium, East Berlin, Germany, April 19-22, 1988.

Attended Supervisor Management Training Session in Bethesda, MD, April 16-20, 1988.

Invited speaker at the ASM Meeting, Miami, Florida, May 8-13, 1988.

Invited speaker at the UC/UA Infectious Disease Meeting, Intermeere, British Columbia, Canada, June 11-16, 1988.

Organized (with Dr. Munoz) the International Workshop on Bordetella pertussis held at Rocky Mountain Laboratories, August 18-20, 1988.

Attended the Cold Spring Harbor Vaccine Meeting, Cold Spring Harbor, NY, September 14-18, 1988.

Invited speaker at the Fifth International Symposium on Pertussis, Copenhagen, Denmark, September 22-23, 1988.

Reviewed manuscripts for Journal of Bacteriology and Infection and Immunity.

Dr. Willy Burgdorfer

Invited to present lecture at Lyme Disease Workshop organized by Roche Laboratories, Washington, D.C., February 29-March 1, 1988.

Recipient of the Robert Koch Golden Medal Award for the discovery of the Lyme disease spirochete, Borrelia burgdorferi, May 19, 1988.

Invited to present lecture at workshop on Lyme disease organized by the Lyme Borreliosis Foundation, Inc., Hartford, CT, June 9, 1988.

Nominated Scientific Director to the Board of the Lyme Borreliosis Foundation, Inc., June 9, 1988.

Participated as instructor at the 1988 Acarology Program of the Ohio State University, Columbus, OH, June 20-25, 1988.

Invited to present lecture "Lyme Disease: the challenge of diagnosis" at the Ohio State Health Department, Columbus, Ohio, June 23, 1988.

Invited to present papers on vector-borne diseases of veterinary importance and on risk factors and their prevention at the 125th Annual Meeting of the AVMA, Portland, OR, July 18-19, 1988.

Presented papers at the 43rd Annual Northwestern Conference on Diseases of Nature Communicable to Man, Boise, ID, August 15-17, 1988.

Invited to deliver keynote address at the 10th Anniversary of the Institut Central des Hopitaux Valaisans, Sion, Switzerland, September 8-9, 1988.

Interviewed by Public Broadcasting System (PBS) and 20-20 TV program on Lyme disease and professional accomplishments.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00071-17 LPB

PERIOD COVERED

October 1, 1987, to September 30, 1988

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Studies on Pertussigen

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: John J. Munoz

Acting Head

LPB, NIAID

OTHERS: Marius Peacock

Biol. Lab. Tech. (Micro)

LPB, NIAID

COOPERATING UNITS (if any)

William Black (Emory University), Denise Monack and Stanley Falkow (Stanford University)

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Immunopathology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

1.6

PROFESSIONAL

.8

OTHER:

.8

CHECK APPROPRIATE BOXES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The actions of pertussigen (pertussis toxin (Ptx)) on the IgE-containing spleen cells of C57Bl/10SCN mice, and on the promotion of protective activity of vaccines made from Bordetella species were studied. Mice receiving Ptx and 1 mg of egg albumin (EA) showed a marked increase in the percentage of spleen cells containing surface IgE. This action was more pronounced in older mice (10 weeks or older) than in younger mice (5-6 weeks old). Since the increase of IgE-containing cells was as high as 40% of the total spleen cells, it seems that the main action of the Ptx was that of increasing the receptors for IgE in the B-cell population rather than just increasing the IgE-producing cells. When Ptx was given alone, only a slight increase of IgE-containing cells was observed, and EA alone did not have any effect on the number of these cells.

Ptx increased strikingly the protective action of vaccines made from B. pertussis strains lacking Ptx, or from strains in which a genetically modified gene for the S1 subunit of Ptx was present. Ptx also enhanced the protective activity of vaccines made from B. paraptussis and B. bronchiseptica, but had little effect on vaccines made from wild-type strains of B. pertussis known to produce Ptx.

The antibody response to surface antigens of Bordetella has been studied. We have shown that the antibodies of the IgG class is not increased. However, the IgE class of antibodies was noticeably increased in vaccines containing Ptx or when Ptx was included in the immunization with vaccines lacking Ptx.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 201 AI 00201-09 LPB
PERIOD COVERED October 1, 1987, to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structural Characterization of Integrated Viral Genomes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Claude F. Garon Chief LPB, NIAID		
OTHERS: Lori L. Petersen Biologist LPB, NIAID Stanley F. Hayes Biol. Lab. Tech. (Micro) LPB, NIAID Dan Corwin Biol. Lab. Tech. (Micro) LPB, NIAID		
COOPERATING UNITS (if any) LVD (Moss), LMM (Martin, Khan), LMO/NCI (T. S. Papas), CDC (Mayer)		
LAB/BRANCH Laboratory of Pathobiology		
SECTION Pathobiology		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.9	PROFESSIONAL: 0.4	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Modern methods of molecular cloning and analysis allow for the detection and amplification of rare DNA sequences such as a single integrated viral segment. Molecular clones of several newly integrated retroviral genomes have been produced in either plasmid or bacteriophage cloning vehicles using recombinant DNA techniques and have been characterized using electron microscope heteroduplex and R-loop methods. Detection of sequence homology even when rearranged or interrupted by intervening cellular DNA is often accurately mappable in the electron microscope using these methods. These studies have not only shown the arrangement of integrated viral sequences within infected host cell DNA, but have also demonstrated the presence and sequence arrangement of certain viral transforming sequences within normal, uninfected host cells as well. These studies have recently been expanded to include a structural determination of the genetic relatedness of AIDS virus isolates from around the world. Unique inverted repeat sequences structurally resembling transposable elements have been identified in the area of retroviral integrations and molecularly cloned for further analysis. The major objective of these studies has been the application of physical and biochemical techniques to assess the influence of integrative position or flanking cellular sequences on subsequent viral function and to define in molecular terms those events which take place during integrative recombination in eukaryotic cell systems.		
17-9		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 201 AI 00402-05 LPB
PERIOD COVERED September 30, 1987 to October 1, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Cloning and Expression of Bordetella Pertussis Toxins		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: Jerry M. Keith, Head, LPB, NIAID Others: K. Vadheim, Guest Worker W. Cieplak, NRC Researcher M. Szulczynski, Visiting Fellow S. Strachen, Guest Worker K. Kaljot, NRSA Researcher All LPB, NIAID S. Northcutt, Microbiologist S. Smith, Microbiologist Y. Lobet, Visiting Fellow D. Brown, Sr. Staff Fellow		
COOPERATING UNITS (if any) AMGEN (W. N. Burnette, V. Mar), Thousand Oaks, CA; National Institutes of Health (H. Sato), Tokyo, Japan		
LAB/BRANCH Laboratory of Pathobiology		
SECTION Molecular Pathobiology		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS 4.83	PROFESSIONAL 2.7	OTHER 2.13
CHECK APPROPRIATE BOXES: ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided) The functional objectives of this project are to investigate genetic molecular structure of pathogens, to define the role of gene products in pathogenic mechanisms and to perform studies directed toward development of vaccines using molecular biology. Our primary emphasis focuses on cloning and expression of genes relevant to the toxic components of <u>Bordetella pertussis</u>. Pertussis toxin is the protective component of the whooping cough vaccine; however, its biological activities may cause the harmful side effects associated with current vaccines. This protein toxin is composed of five dissimilar subunits; the S1 subunit contains an ADP-ribosyltransferase activity responsible for most if not all of the biological activities of the toxin. Past accomplishments of the laboratory include: cloning and sequencing the pertussis toxin operon (patent application filed and licensed for vaccine development); direct expression of toxin subunits in <u>Escherichia coli</u>; and characterization of structure and function relationship between pertussis toxin, pathogenesis, and immunoprotection. Using the results of these studies as a basis for further research, we have identified a region in the S1 subunit that is required for enzymatic activity and that contributes to the formation of a neutralizing antigenic determinant. The gene encoding the S1 subunit was modified in this critical region by site-specific mutagenesis. One of the mutants in which a single amino acid substitution was engineered into the S1 subunit had no enzymatic activity while retaining the protective epitope. The genetic changes responsible for the loss of enzymatic activity are now being engineered into the chromosome of <u>B. pertussis</u> to develop a new strain of the bacteria which will produce a genetically detoxified pertussis toxin. This modified toxin has important potential for use as a component of an acellular vaccine against whooping cough and is the subject of two invention reports and patent applications to be filed this year.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00480-03 LPB.
PERIOD COVERED October 1, 1987, to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pathogen-arthropod Interactions of Vector-borne Diseases Affecting Public Health		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Tom G. Schwan Senior Staff Fellow LPB, NIAID		
OTHERS: Rex E. Thomas IRTA Fellow LPB, NIAID Robert H. Karstens Bio. Lab. Tech. (Micro) LPB, NIAID		
COOPERATING UNITS (if any) Center for Disease Control, Fort Collins, CO (A. M. Barnes, T. Quan); Stanford University, CA (K. McDonough and S. Falkow); University of Maryland (A. Farhang-Azad)		
LAB/BRANCH Laboratory of Pathobiology		
SECTION Arthropod-borne Diseases		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.7	PROFESSIONAL: 1.2	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The objectives of this project are to develop molecular approaches to investigate pathogen-arthropod interactions of vector-borne diseases of human importance in the United States. The first objective was to develop a sensitive DNA hybridization probe to specifically identify <u>Yersinia pestis</u>, the causative agent of bubonic plague, in experimentally infected fleas. This was achieved in collaboration with K. McDonough and S. Falkow at Stanford University. <u>Yersinia pestis</u> infection in individual fleas of three species (<u>Xenopsylla cheopis</u>, <u>Thrassius bacchi</u>, and <u>Diamanus montanus</u>) were detectable using a 1000 base pair restriction fragment cloned from the pesticin plasmid. The same probe also detected <u>Y. pestis</u> in impression smears of spleen and liver of experimentally infected mice. A similar study has begun to develop a DNA probe to detect <u>Rickettsia typhi</u>, the causative agent of murine typhus, in fleas. Protocols for the purification of murine toxin and Fraction 1 antigen of <u>Y. pestis</u> are being developed to provide reagents for alternative methods for the rapid detection of <u>Y. pestis</u> in infected fleas.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00488-02 LPB

PERIOD COVERED

October 1, 1987, to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ultrastructural Analysis of Antigenic Determinants in Pathogens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Claude F. Garon Chief LPB, NIAID

OTHERS:	Stanley F. Hayes	Bio. Lab. Tech. (Micro)	LPB, NIAID
	Dan Corwin	Bio. Lab. Tech. (Micro)	LPB, NIAID
	Willy Burgdorfer	Scientist Emeritus	LPB, NIAID
	David W. Dorward	Staff Fellow	LPB, NIAID
	Lori L. Petersen	Biologist	LPB, NIAID
	Tom G. Schwan	Sr. Staff Fellow	LPB, NIAID

COOPERATING UNITS (if any)

LPVD, RML, NIAID; LMSF, RML, NIAID
Department of Medical Microbiology - Stanford University School of Medicine
Department of Microbiology and Immunology - University Rochester Medical Center

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Pathobiology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

2.5

PROFESSIONAL

1.1

OTHER:

1.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

This project serves to consolidate and to expand our efforts at identifying, localizing, and characterizing genes and gene products which are important in host pathogen relationships. While we have used colloidal gold and ferritin labeling to identify important cellular components which are located on the surface of various pathogens, new techniques of cryofixation, cryoultramicrotomy and immuno-labeling will allow precise localization of important antigens which may be located inside the cell. A broad range of molecular techniques will allow tracking of antigens through synthesis, processing and genetic control modulation. We have recently developed procedures for laboratory synthesis of uniform, precisely-sized colloidal gold markers which, when coupled with functional and specific antibody molecules, will allow for multiple antigen location and subsequent ultrastructural analysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00489-02 LPB
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Purification and Biochemistry of Molecularly Cloned <u>Bordetella</u> Proteins		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Jerry M. Keith, Head, LPB, NIAID		
Others: W. Cieplak NRC Researcher S. Strachen Guest Worker Y. Lobet Visiting Fellow S. Smith Microbiologist M. Szulczynski Visiting Fellow K. Kaljot NRSA Researcher D. Brown Sr. Staff Fellow All LPB, NIAID S. Northcutt Biologist		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Pathobiology		
SECTION Molecular Pathobiology		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 2.75	PROFESSIONAL: 2.15	OTHER: 0.6
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.) Development of new and safer whooping cough vaccines is a major priority of health policy organizations and vaccine manufacturers. Evidence accumulating from clinical trials of acellular pertussis products strongly indicates that pertussis toxin will be a necessary, and perhaps sufficient, component of any new vaccine. Anticipating the need for purer and non-reactogenic reagents, we have cloned and recombinantly expressed the genes for each of the toxin component proteins in <u>Escherichia coli</u>. These nonfusion recombinant polypeptides are synthesized to very high levels as cellular inclusion bodies, greatly facilitating their isolation. This project involves purification and characterization of these protein antigens produced by the host system. In many instances, these expressed proteins represent up to 40% of the total cell protein; however, these proteins are often insoluble and form inclusion bodies in the <u>E. coli</u> cell. Various techniques were developed to solubilize these proteins. We have succeeded in purifying various versions of subunits S1, S2, and S4. Manipulation of the gene segment encoding the A protomer (S1 subunit) has permitted identification of regions possessing both immunodominant and immunoprotective epitopes as well as sites critical for enzymatic activities. We expect that our recombinant reagents will allow us to develop a stable, safe, and efficacious acellular vaccine against pertussis.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00490-02 LPB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Animal Protection Studies Using Synthetic and Recombinant Pertussis Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Jerry M. Keith, Head Molecular Pathobiology, LPB, NIAID

Others:	K. Kaljot	NRSA Researcher	LPB, NIAID
	S. Northcutt	Biologist	LPB, NIAID
	W. Cieplak	NRC Researcher	LPB, NIAID
	S. Strachen	Guest Worker	LPB, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Molecular Pathobiology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

2.08

PROFESSIONAL

1.15

OTHER

0.93

CHECK APPROPRIATE BOXES)

- | | | |
|---|--|---|
| ☐ (a) Human subjects | ☐ (b) Human tissues | ☒ (c) Neither |
| ☐ (a1) Minors | | |
| ☐ (a2) Interviews | | |

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

This project involves performing studies directed towards the development of vaccines using recombinant DNA technology to synthesize immunogenic peptides from microbial agents. The objective is to identify specific natural or genetically modified proteins which when used as immunogens will protect against challenge by *Bordetella pertussis*, the causative agent of whooping cough. These antigens will then be considered for use in a new generation component vaccine. We are presently working on pertussis toxin, a protein consisting of five subunits, which is a major protective immunogen in whooping cough vaccines. Our initial effort involves evaluating individual subunits of pertussis toxin produced synthetically using recombinant DNA technology. Our earliest results suggested that the standard mouse lethal intracerebral challenge (ic) model was incapable of reliably identifying protective pertussis components and was variably reliable in demonstrating protection with a commercially available whole cell vaccine. By modifying the ic challenge to include a small dose of active pertussis toxin, which is incapable of stimulating immunoprotection when administered alone, we have been able to consistently demonstrate protection following immunization with both purified components and recombinant component subunits. In the 31 experimental groups studied so far, the first 15 did not utilize active pertussis toxin. In those experiments, immunization with recombinant pertussis toxin subunits S1 and S2 produced in *E. coli* did not yield protection which was greater than that provided by vehicle alone. Following inclusion of active toxin in the protocol, immunoprotection by S1 and S2 reached 70% of the level provided by the whole cell vaccine which served as a positive control. Deletion and substitution mutants of the S1 subunit are currently being tested to identify the epitopes associated with protection from lethal challenge. In addition, S1 based peptides are being synthesized to test their protective potential.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 000491-02 LPB
PERIOD COVERED October 1, 1987, to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Role of antibodies of IgE isotype in immunologically mediated diseases.		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: John J. Munoz Acting Head LPB, NIAID		
OTHERS: Marius Peacock Bio. Lab. Tech. (Micro) LPB, NIAID		
COOPERATING UNITS (if any) Richard Buller (LPVD, Rocky Mountain Laboratories) LAB/BRANCH Laboratory of Pathobiology SECTION Pathology INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: .4	PROFESSIONAL: .2	OTHER: .2
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Most of the work in this project has been devoted to developing reliable reagents to measure IgE with specificity to the antigens used in this project. In addition, some efforts have been made to develop hybridomas producing IgE with specificity to a desired antigen. Specific antisera for the reliable detection of IgE have been obtained, but hybridomas producing IgE have not been yet developed. Presently, enrichment techniques to concentrate the numbers of IgE-producing cells before fusing with tumor cells are under investigation. Employing the IC challenge to detect immunity to experimental pertussis in mice, it has been shown that vaccines made from <i>Bordetella pertussis</i> containing pertussigen (pertussis toxin (Ptx)) stimulate the production of antibodies of the IgE isotype with specificity to surface antigens of <i>B. pertussis</i> . Strains that do not produce Ptx do not stimulate IgE antibodies. Antisera containing IgE antibodies to surface antigens protected mice from IC challenge, while those without it did not protect mice. These experiments are under further study to settle whether the IgE antibodies are indeed important in the protection of mice to IC challenge.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00492-02 LPB

PERIOD COVERED

October 1, 1987, to September 30, 1988

TITLE OF PROJECT (90 characters or less. Title must fit on one line between the borders.)

Molecular Basis for Infection by *Borrelia burgdorferi*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)

PI: Tom G. Schwan

Sr. Staff Fellow

LPB, NIAID

OTHERS: Willy Burgdorfer

Scientist Emeritus

LPB, NIAID

Stephanie Czub

Visiting Fellow

LPB, NIAID

Merry E. Schrupf

Bio. Lab. Tech. (Micro)

LPB, NIAID

Robert H. Karstens

Bio. Lab. Tech. (Micro)

LPB, NIAID

COOPERATING UNITS (if any)

Southampton Hospital, Southampton, NY (A. MacDonald)

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Arthropod-borne Diseases

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

2.8

PROFESSIONAL

1.3

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The objectives of this project are to (1) determine if genes present on plasmid DNA of *Borrelia burgdorferi*, the causative agent of Lyme disease, control infectivity, (2) characterize these plasmids, (3) clone the infectivity genes and express those components that promote borrelia infection in mammals, and (4) determine immunogenic properties of components responsible for infection. The reduction in the number of detectable plasmids with the loss of infectivity suggests that gene(s), encoding for components related to infectivity, may be present on one or more of these extrachromosomal elements. A 7.6 kilobase (kb) pair circular plasmid has been identified as a strong candidate for regulating infection. Various restriction fragments from this plasmid have been cloned and are currently being used as probes for identifying infectious strains. These probes will also be useful in demonstrating the true fate of the 7.6 kb plasmid, which appears to be lost from the spirochete as a result of serial in vitro cultivation. Investigations concerning antigenic variation of *B. burgdorferi* in vitro and in vivo using a mouse model are continuing. Both monoclonal and monospecific polyclonal antibodies are being developed to help define antigenically variable determinants.

Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
1988 Annual Report
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Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1987 to September 30, 1988

RESEARCH HIGHLIGHTS

Strand specific in situ hybridization identified lymph node germinal centers as sites of Aleutian disease parvovirus replication and distinguished these cells from sites of virus accumulation in the reticuloendothelial system of infected mink.

Complete DNA sequence and transcription maps obtained for Aleutian disease virus of mink. Comparison of stains suggested sites influencing viral virulence in vivo.

Envelope gene of neurotropic mouse retrovirus is responsible for ability to infect neuronal and glial cells. Accumulation of unintegrated viral DNA in infected cells may explain persistent virus expression in non-dividing neuronal cells.

Unique endogenous retroviral envelope genes were shown to be expressed on erythroid and granulocytic precursor cells of normal mice.

Genetically controlled resistance of mice to rabies virus was shown not to be mediated by interferon.

Hormone-mediated alteration of female protein levels in hamsters was able to influence normal rate of deposition of amyloid in liver and other target organs.

Clones of mouse neuroblastoma cells chronically infected with the scrapie agent were compared to uninfected clones for prion protein expression and biosynthesis. No proteinase K-resistant prion protein was detected, suggesting this form may not be required for expression of scrapie agent.

Variants of equine infectious anemia virus, a horse lentivirus, were found to be distinguishable by host cell tropism in vitro. This tropism may play an important role in pathogenesis in vivo.

Retroviral recombinant virus identified in vitro as probable intermediate stage in generation of oncogenic recombinant MCF retroviruses.

Focal immunoassay for HIV infectivity was developed. The assay can detect one infected cell out of one million cells, and is capable of scoring most wild-type HIV isolates.

Immune response gene in the H-2I region controlling the response of mice to Friend retroviral envelope protein has been shown to act by controlling presentation of envelope antigen to specific T lymphocytes. This genetic effect strongly influences induction of protective immunity to Friend virus challenge.

Variation in retroviral pathogenesis correlated with a single amino acid change in the retroviral envelope protein.

Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1987 to September 30, 1988

ADMINISTRATIVE REPORT

The following staff changes occurred at LPVD this past year:

Arrivals:

- Dr. Markus Czub, Visiting Fellow, from Berlin, West Germany, working with Dr. John Portis.
- Dr. Shiro Mori, Visiting Fellow, from Sendai, Japan, working with Dr. Marshall Bloom.
- Dr. Chiaki Ishihara, Guest Researcher, on sabbatical from Sapporo, Japan, working with Dr. Bruce Chesebro.
- Dr. Ray Monk, Senior Staff Fellow, from Cell Science Center, Lake Placid, New York, working with Dr. John Coe.
- Dr. Ditte Tornehave, Visiting Associate, from Odense, Denmark, working with Dr. John Coe.
- Dr. William Lynch, IRTA, from Cornell University, working with Dr. John Portis.
- Dr. Kathy Neary, IRTA, from Yale University, working with Dr. Bruce Chesebro.
- Dr. Wu Wei, Special Volunteer, from the People's Republic of China, working with Dr. Marshall Bloom.

Departures:

- Dr. Soren Alexandersen, Visiting Associate, taking a position in the Department of Veterinary Pathology in Copenhagen, Denmark.
- Dr. Susan Carpenter, Staff Fellow, taking a position at the Department of Microbiology, School of Veterinary Medicine, Iowa State University, Ames, Iowa.
- Summer students were Jennifer Van Horn from Amherst College, Amherst, MA, and David Stokesberry from Oklahoma State University, Stillwater, OK.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00072-17 LPVD
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Role of Host and Viral Factors in Resistance to Rabies Virus Infection in Mice		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	D. L. Lodmell	Scientist Director LPVD, NIAID
Others:	None	
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The principal objective of these studies is to determine host and viral factors that influence the genetically-controlled resistance of inbred mouse strains to street rabies virus (SRV). Studies concerning the importance of interferon induced within the central nervous system (CNS) during infection as a mechanism responsible for resistance have been completed. The interferon was characterized as a typical viral-induced pH 2.0 stable type I interferon with α and β specificity. Furthermore, it was determined that the high concentrations of interferon-α/β that were induced by SRV in the CNS of susceptible A/WySnJ mice were ineffective in inhibiting viral replication. The 100% survival of SRV-infected resistant SJL/J mice following neutralization of interferon within their CNS with high concentrations of rabbit anti-mouse interferon-α/β suggested that the minimal amount of interferon present in the CNS during infection was not the mechanism responsible for their innate resistance to ip-inoculated SRV. Studies concerning the significance of cellular immunity have progressed with the development of an 8 hr assay that measures specific cytotoxic T-cell killing. Maximum killing occurs following a 5 day <u>in vitro</u> incubation of 1×10^6 spleen cells with 1×10^5 ^{137}Cs-irradiated rabies virus-infected stimulator cells; effector to target (E:T) ratios of 10 and 5 result in >70% and >40% specific lysis, respectively. The assay has been simplified by using P815, EL-4 and mouse neuroblastoma target cells persistently-infected with ERA virus. We have demonstrated specific T-cell cytotoxicity with lymphocytes from susceptible A/WySnJ and C57BL/6J mice and resistant DBA/2J and BALB/cByJ mice. Preliminary results suggest that maximum cytotoxic T-cell activity occurs seven days after ip-infection with ERA virus grown in CER cells. Mice infected with similar concentrations of SRV, CVS, and ERA viruses inoculated ip. as 10% mouse brain suspensions have minimal T-cell activity. We have been unable to demonstrate specific T-cell killing in an 8 hr assay with uncultured lymphocytes. The emphasis of these studies will be to continue investigating the importance of cytotoxic T cells in murine resistance to SRV infections.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00074-16 LPVD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetically Controlled Mechanisms of Recovery from Friend Virus-Induced Leukemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	B. Chesebro	Chief	LPVD, NIAID
Others:	M. Miyazawa	Visiting Associate	LPVD, NIAID
	R. Morrison	Sr. Staff Fellow	LMSF, NIAID

COOPERATING UNITS (if any)

Drs. B. Moss and P. Earl, LVD, NIAID, Bethesda, MD

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

2.3

PROFESSIONAL

1.6

OTHER:

.7

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The goal of this project is to understand genetically controlled mechanisms involved in host defense against retrovirus-induced disease. In this work we have been studying both spontaneous recovery and generation of protective immunity using Friend virus complex (FV)-induced erythroleukemia in mice as a model for human retroviral diseases including AIDS and certain leukemias.

Previous experiments indicated that both susceptibility to virus-induced immunosuppression and lack of ability to spontaneously recover from leukemia were influenced by the H-2D subregion. More recent data now shows that the predominant genetic influence on generation of protective immunity is mediated by the H-2I subregion. This was specifically documented in mice immunized by inoculation with live recombinant vaccinia virus expressing the FV envelope gene. Our latest experiments indicate that the H-2I region regulates ability to prime T lymphocytes with FV envelope antigens. The H-2I gene influences this immunological priming by controlling the ability of macrophages and other cells to present FV envelope antigens correctly to the specific T lymphocytes. This mechanism was directly demonstrated in vitro and antigen presentation could be inhibited only by appropriate anti-H-2I monoclonal antibodies. Similar genetic regulatory influences are likely to exist in humans and may affect potency of vaccines being developed against human retroviruses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00085-11 LPVD
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pathogenesis of Aleutian Disease Virus Infection		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	M. E. Bloom	Medical Officer LPVD, NIAID
Other:	S. Alexandersen S. Mori	Visiting Associate Visiting Fellow LPVD, NIAID LPVD, NIAID
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.4	0.5
CHECK APPROPRIATE BOXES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) The scope of this project is the study of infections of mink with the Aleutian mink disease parvovirus (ADV). In the past year we have extended the use of strand-specific in situ molecular hybridization to define sites of ADV replication and sequestration in experimentally infected adult mink. In infected adult mink, target cells for ADV replication could be found only in lymphoid organs. These cells (up to 2 % of total) were located in the centers of germinal centers and scattered throughout the mesenteric lymph node cortex; a distribution suggestive of cells of the B-cell lineage. However, the amounts and ratios of replicative intermediates (RF DNA, mRNA and vDNA) in each infected cell dramatically differed from the permissive cell-virus systems we previously studied; the levels of all 3 were damped by at least a factor of 10 and the amount of RF was disproportionately decreased. These results have led us to conclude that infection in these target cells was being restricted by some mechanism. Furthermore, as infection progressed to a chronic state, cells replicating virus became undetectable, suggesting that the number was either extremely low ($<1/10^3$) or that the restriction was progressively more severe. A restriction on viral replication and transcription in ADV infected lymphoid cells might provide a mechanism for the development of immune disorders and for the maintenance of persistent infection. Large amounts of virions were sequestered in the periphery of germinal centers in a distribution very similar to that of processed antigen, possibly providing an explanation for the extreme antiviral antibody response observed in these animals. Antiviral antibody may be important in the development of persistent infection, because treatment of ADV infected mink kits with specific antibody converted the highly permissive infection of the alveolar type II cells into a restrictive one similar to that described above.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00086-11 LPVD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the colons)

Pathogenesis of Diseases Induced by Non-Oncogenic Retroviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J.L. Portis Medical Officer LPVD, NIAID

Others: Marcus Czub Visiting Fellow LPVD, NIAID

COOPERATING UNITS (if any)

Dr. WG Stroop, Neurovirology Research Lab, Univ Utah Sch Med, Salt Lake City, UT;
Dr. R. Ziegler, Univ Minn Sch Med, Dept Microbiology, Deluth, MN

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

2.0

PROFESSIONAL

1.0

OTHER:

1.0

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

There is mounting evidence that some neurodegenerative diseases of man are associated with human retrovirus infection. The mechanisms by which retroviruses cause neurologic disease are not understood. This project focuses on the study of a murine neurodegenerative disease that is caused by a retrovirus (WM-E) which is indigenous to certain wild mouse populations in California and Asia. The virus is transmitted congenitally through maternal milk as well as horizontally in the semen through sexual intercourse. The pathology of this disease consists of the progressive death of motor neurons in the spinal cord, midbrain and cerebellum and in this regard resembles amyotrophic lateral sclerosis of man. The primary goals of the project are to define the specific targets for the virus in the CNS, the mechanism of virus spread, host and viral factors which influence virus spread within the CNS and the mechanism of the cytolytic effect of this virus on neurons. Previous studies revealed that extravascular spread of WM-E within the CNS was mediated by the viral envelope gene. We now have evidence that the viral *env* gene also endows this virus with a unique tropism for neuronal as well as astrocytic cell lines in vitro, providing compelling evidence for the existence of specific receptors in the CNS for WM-E. We have recently found that large amounts of unintegrated DNA persist long after infection of fibroblastic cells in vitro. In this respect WM-E resembles the lentiviruses and cytopathic avian leukosis viruses in which similar accumulation of unintegrated viral DNA has been observed. It is thus possible that unintegrated WM-E DNA may persist in neurons in the CNS allowing viral gene expression without viral DNA integration. Further work in this area will involve studies of purified cell populations from the CNS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00199-08 LPVD

PERIOD COVERED

October 1, 1987, to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunobiology of Aleutian Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. E. Race Research Veterinarian LPVD, NIAID

Others: M. E. Bloom Medical Officer LPVD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00260-07 LPVD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (50 characters or less. Title must fit on one line between the borders.)

Role of Endogenous and Recombinant Retroviruses in Leukemia and Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator) (Name, title, laboratory, and institute affiliation)

PI:	B. Chesebro	Chief	LPVD, NIAID
Others:	L. Evans	Senior Staff Fellow	LPVD, NIAID
	F. Hayes		LPB, NIAID

COOPERATING UNITS (if any)

Dr. L. D'Auriol, Hopital St. Louis, Paris, France
M. Sitbon, Hopital Cochin, Paris, France

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1

PROFESSIONAL

0.1

OTHER:

0.9

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
☐ (b) Human tissues
☒ (c) Neither

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

The goal of this work is to study mechanisms of pathogenesis of murine retroviruses. Previous studies showed that two closely related variants of Friend murine leukemia viruses differed in their ability to induce hemolytic anemia and splenomegaly shortly after inoculation into newborn mice. In vitro construction of recombinants between these strains demonstrated that this difference was due to differences in the viral envelope gene. DNA sequencing has revealed only 2 sequence differences between these strains, and site-specific mutagenesis has now identified a single amino acid responsible for this alteration in pathogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00262-07 LPVD
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Role of Pentraxins in Acute and Chronic Pathology		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	J. E. Coe	Medical Officer LPVD, NIAID
Others:	D. Tornehave	Visiting Associate U of Odense, Denmark
COOPERATING UNITS (if any) See attached page		
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.3	2.3	1.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Steroid hormones produce some remarkable effects in hamsters. The hepatic synthesis of a pentraxin protein, female protein (FP), is controlled by sex steroids in the Syrian hamster. The resulting high serum levels in female hamsters is directly associated with another unique phenomenon, i.e., a sex limited expression of amyloidosis which predominantly affects female hamsters. FP is a constituent of hamster amyloid and the presence of amyloidosis (incidence and amount) was diminished by administration of exogenous steroids which depressed hepatic synthesis of FP. The role of FP in hamster amyloidosis is thought to be analogous to that of its homolog, serum amyloid P component (SAP), in human amyloidosis; that is, SAP incorporation is depicted as a secondary event after formation of fibrils, the main constituent of amyloid. These observations in hamsters indicate that the P component homolog (FP) has a primary role in hamster amyloidosis. In contrast to Syrian hamsters, estrogen (DES) administration induces an opposite pentraxin response in Armenian hamsters, as FP synthesis is inhibited. Also, estrogens induce an acute toxicity (icterus) in Armenian and Chinese hamsters (but not Syrian hamsters), and these effects are mediated by estrogen receptors. Finally, chronic estrogen injections can induce appearance of multiple tumors in the livers of both male and female hamsters.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00263-07 LPVD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT: 80 characters or less. Title must fit on one line between the borders.

Structure and Function of the ADV Genome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: M. E. Bloom Medical Officer LPVD, NIAID

Others: Soren Alexandersen Visiting Associate LPVD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MANY-YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOXES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The purpose of this project is the study of genome structure and function of the Aleutian mink disease parvovirus (ADV). In the past year, we confirmed the sequence of the ADV-G strain of ADV and derived molecular clones containing the 5'-palindrome (right hand terminus). Detailed computer analysis of the ADV-G sequence has revealed that the 3'-terminal palindrome could assume a Y-shaped configuration, similar to that of the other parvoviruses, but was less stable thermodynamically. Furthermore, the overall homology to other parvoviruses was <50%, but short conserved amino acid regions were noted in both the left and right major open reading frames (ORFs). Two allegedly conserved regions in the right ORF were not found in ADV. ADV-G was 97.5% related to the virulent ADV-Utah 1 in a comparison encompassing 76% of the genomes, but we observed a short hypervariable region in the right ORF in which 8 of 11 amino acid residues varied. This short sequence may correspond to regions in other parvoviruses that govern host-range and virulence, and, thus, may be responsible for the pathogenicity characteristics of different ADV strains. A molecular clone of ADV-G that contains the full sequence, including both termini, has been constructed and is now being tested for infectivity. The transcription program of ADV-G has been analyzed in detail using Northern blotting, cDNA cloning, DNA sequencing, and primer extension. ADV had 5 polyadenylated RNA transcripts of 4.3 (R1), 2.8 (R2), 2.8 (R3), 1.1 (RX), and 0.85 (R2') kb. Each mRNA had ORFs suggestive of bona fide protein coding sequences, initiating from promoters at 3 map units (R1, R2, R2', and RX) or at 36 map units (R3) and terminating at polyadenylation sites at map unit 53 (R2' and RX) or map unit 92. A transcript with characteristics of RX has not been previously described for the parvoviruses. R3 almost certainly coded for the capsid proteins (p85 and p75); R1, for the largest nonstructural proteins (p71); and the others, for 1 or more additional nonstructural proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00264-07 LPVD
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structure and Function of Endogenous Retroviruses Expressed During Differentiation		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	J.L. Portis	Medical Officer LPVD, NIAID
Others:	R. Buller B. Chesebro	Staff Fellow Chief LPVD, NIAID LPVD, NIAID
COOPERATING UNITS (if any) Dr. Gary Van Zant, Dept. Molec. and Cell Biology, Texas Tech Univ., Lubbock, TX.		
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) The central theme of this project involves the role of endogenous retroviral gene products in the genetic resistance to retrovirus induced leukemogenesis exhibited by certain strains of mice. We previously demonstrated that an endogenous retroviral <u>env</u> gene with two alleles is linked to the Rmcf locus on chromosome 5. One allele encodes an endogenous gp70 structurally related to that of recombinant MCF viruses, and expression of this gene in vitro results in the restriction of fibroblastic cells to infection by MCF viruses. This resistance appears to be mediated by receptor blockade (viral interference). In genetic backcross experiments this gene was shown to mediate resistance to erythroleukemia induced by neonatal inoculation with non-defective Friend murine leukemia virus (F-MuLV). Using Fluorescence Activated Cell Sorting techniques along with in vitro functional assays of hematopoietic progenitor cells we have now defined the cells in the mouse which express this gene. Expression appears to be initiated within the pool of multipotential stem cells (CFU-S) and is detectable on the majority of erythroid progenitors (BFU-E and CFU-E) as well as granulocytic precursors (GM-CFU) but is shut off at later stages of myeloid differentiation. The gene also is expressed by lymphoid progenitors which are resident in low numbers in the thymus but as in the myeloid compartment is not expressed by mature lymphocytes. This pattern of expression strongly suggests that this endogenous retroviral <u>env</u> gene mediates resistance to erythroleukemia by interfering with infection of primitive erythroid progenitors (presumably BFU-E) by recombinant MCF viruses generated as a consequence of infection by F-MuLV. This endogenous retroviral gene appears to define a region of chromosome 5 which is transcriptionally active during early stages of hematopoietic lineage commitment. Of interest is the close association of this gene with the W^V locus on chromosome 5 which is involved in hematopoietic stem cell function. In order to further define its structure and function we are cloning this retroviral gene from a C-DNA library.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00265-07 LPVD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (50 characters or less. Title must fit on one line between the colons.)
Immunobiology of Scrapie Virus Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. E. Race	Research Veterinarian	LPVD, NIAID
Others:	B. Caughey	Staff Fellow	LPVD, NIAID
	B. Chesebro	Chief	LPVD, NIAID

COOPERATING UNITS (if any)

Dr. A. Haase, Chief, Dept. Microbiology, University of Minnesota, Minneapolis, MN; Dr. M. Buchmeier, Dept. of Immunology, Scripps Clinic and Research Foundation, La Jolla, CA.

LABORATORY

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MANYEARS.

3.2

PROFESSIONAL

2.2

OTHER:

1.0

CHECK APPROPRIATE BOXES)

- | | | |
|---|--|---|
| ☐ (a) Human subjects | ☐ (b) Human tissues | ☒ (c) Neither |
| ☐ (a1) Minors | | |
| ☐ (a2) Interviews | | |

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Scrapie is a naturally occurring spongiform encephalopathy of sheep and goats which causes clinical and pathological changes similar to those of Creutzfeld-Jakob and Kuru diseases of man. A unique protein called prion protein (PrP) has been found to be a major component of purified samples containing scrapie infectivity and is believed by some people to be the infectious agent. It is just as possible though that the protein accumulates as a secondary by-product of the disease in vivo. To discriminate between these possibilities we studied PrP biosynthesis *in vitro* in scrapie infected and uninfected neuroblastoma cells using pulse-chase labelling experiments. No differences in PrP biosynthesis were found, and this suggested a secondary role for PrP in scrapie pathogenesis.

Because animal tissues have not provided satisfactory substrates for the biochemical analysis or isolation of the scrapie agent, we have continued to develop and improve an *in vitro* scrapie infected cell culture system which we described before. Scrapie infected cultures were derived from single positive cells and contained 50-100 fold more agent than was found in uncloned cultures. By frequency analysis nearly every cell in expanded cultures continued to contain scrapie agent. Cell dose-infectivity relationships were studied and a standard curve was developed which allowed various cultures to be compared, thus significantly reducing the numbers of animals needed to titrate various samples. These cultures will be used to test methods for agent purification.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00266-07 LPVD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (30 characters or less. Title must fit on one line between the borders.)

Genetic Structure of Murine Retroviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator.) (Name, title, laboratory, and institute affiliation)

PI: L. H. Evans Senior Staff Fellow LPVD, NIAID

Others:

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

2.0

PROFESSIONAL

1.0

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The effects of genetic alterations of retroviruses include the generation of variant viruses which infect different hosts; viruses which are capable of infecting different tissues in the host; and virus variants which exhibit enhanced pathogenicity compared to the "wild type" virus. Such alterations have been documented with nearly all groups of retroviruses, including avian, murine, equine, and most recently with HIV, the etiological agent of AIDS. This project is largely focused on the genetic alteration of murine leukemia viruses (MuLVs), principally with regard to alterations due to recombination and the role of this process in disease induction. Previous studies include the identification of a novel recombinant virus which provided a detailed model for the generation of oncogenic viruses in mice which exhibit a high incidence of leukemia. In our current studies we have demonstrated the generation of a variant virus in cell culture by spontaneous recombination between viruses postulated as intermediates in our model. The variant virus exhibits in vitro properties indistinguishable from those of oncogenic recombinant viruses isolated from mice, and is currently being tested for in vivo oncogenicity. Other studies have demonstrated that the viral envelope protein strongly influences the tissue in which viruses replicate at early times after inoculation, whereas viral sequences which control the rate of transcription influence tissue-specific expression at later times. These results have been extended to show that early pathological events in leukemia induction are influenced by the viral envelope protein, while the type of leukemia which subsequently develops at later times is influenced by transcriptional control elements. Many of these studies have been greatly facilitated by the development of a retrovirus assay on live cells using monoclonal antibodies. To further extend the usefulness of this assay we have developed a broadly reactive monoclonal antibody directed at the envelope proteins of MuLVs. The antibody facilitates the assay of a very broad spectrum of MuLVs, including the amphotropic MuLVs for which no monoclonal antibodies directed at the envelope protein have been described.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00418-05 LPVD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunobiology of Equine Infectious Anemia Virus, a Retrovirus Model for AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: B. Chesebro Chief LPVD, NIAID

Others: S. Carpenter Staff Fellow LPVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.2

PROFESSIONAL:

1.1

OTHER:

.1

CHECK APPROPRIATE BOXES:

- ☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Equine infectious anemia virus (EIAV) is morphologically, genetically, and serologically related to human immunodeficiency virus (HIV) and thus provides a useful model for the study of virus-host interactions in lentivirus-induced disease. Characteristic of equine infectious anemia (EIA) is the episodic nature of clinical disease which is associated with the appearance of novel viral variants. This project is concerned with elucidating the factors important in the generation and selection of these viral variants and in understanding the role of variation in lentivirus persistence and pathogenesis. Our previous work suggested that early in the course of disease, EIAV variants may be selected in vivo by non-immunological mechanisms. We have therefore initiated studies on the in vitro host cell tropism of various strains of EIAV and its possible correlation with viral pathogenicity. Virus isolates which were selected for in vitro replication in continuous cell lines demonstrated a 1,000-10,000-fold reduction in viral replication in primary horse monocyte cultures (HMC) as compared to replication in continuous cell lines. Moreover, this marked reduction of viral replication in horse monocytes, believed to be the target cell for EIAV replication in vivo, was associated with a loss of viral virulence. This suggests that levels of viral replication in in vitro HMC may be a correlate of in vivo pathogenicity. Thus, we have increased our efforts to isolate a replication-competent molecular clone of EIAV which could serve as a basis for understanding structural viral features important in viral pathogenicity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00468-03 LPVD
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biology of Human AIDS Retrovirus		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	B. Chesebro	Chief LPVD, NIAID
Others:	None	
COOPERATING UNITS (if any)		
Dr. T. Folks, LIG, NIAID, Bethesda, MD; Dr. B. Moss, LVD, NIAID, Bethesda, MD		
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	0.3	0.8
CHECK APPROPRIATE BOX(ES)		
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither		
☐ (a1) Minors		
☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)		
The goal of this work has been to develop a sensitive quantitative in vitro focal immunoassay for human immunodeficiency virus (HIV) and HIV-infected cells. This has been approached by inserting the human CD4 gene into human cell lines. Clones with high CD4 expression were then used as targets for HIV infection which was detected by HIV-specific antibodies with indirect immunofluorescence or immunoperoxidase methods. The assay was shown to be HIV-specific by using monoclonal antibodies specific for HIV envelope or core proteins; however, polyclonal human anti-HIV serum was also effective. The assay was highly sensitive, capable of detecting a single infected cell out of one million uninfected cells. Furthermore, the assay was capable of detecting most wild-type HIV strains isolated from patients. Future experiments are aimed at using the assay to study HIV expression in cells of AIDS patients.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00524-01 LPVD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (30 characters or less. Title must fit on one line between the colons)

Immunologic Factors in Susceptibility and Resistance to Rabies Virus in Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator) (Name, title, laboratory, and institute affiliation)

PI: L. L. Perry Expert

LPVD, NIAID

Others:

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

2.0

PROFESSIONAL

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The primary goal of this project is to define immunological mechanisms of susceptibility and resistance to rabies virus in inbred strains of mice. A model system for analysis established by Dr. Donald Lodmell of this division is being used to characterize the cellular and humoral immune response of susceptible (A.SW, A/WySn) and resistant (SJL) strains of mice during infection with street rabies virus (SRV), a highly virulent strain that is lethal in susceptible hosts. Preliminary studies revealed a diminished *in vitro* T cell proliferative response in A.SW^S as compared to SJL^r hosts which was not due to defective presentation of viral antigens, as antigen-pulsed macrophages from A.SW^S or SJL^r mice stimulated immune SJL^r T cells equally well. A gradual decrease in spleen size in A.SW^S but not SJL^r hosts was also noted. Attempts to define the basis for these observations led to immunofluorescent analyses of spleen lymphocyte subsets in these two strains of mice using a fluorescence activated cell sorter. FITC or phycoerythrin conjugated monoclonal antibodies (mAb) specific for T cells (anti-Thy 1.2 mAb), B cells (anti-kappa mAb), or I-A⁽⁺⁾ cells (10-3.6 mAb) were used to stain spleen cells derived from mice at various times after SRV infection. Results indicate a dramatic (2-5 fold) decrease in all populations defined by these surface markers from two to seven days after infection in A.SW^S but not SJL^r hosts. Anti-SRV antibody responses, detected by RIA on intact virus using isotype-specific secondary antibody reagents, are also minimal in the susceptible animal. Efforts to protect A.SW^S mice from rabies lethality by adoptive transfer of immune SJL^r histocompatible cells have been unsuccessful, perhaps due to a paucity of IL-2 and other essential lymphokines in the infected A.SW^S recipient. It has also been possible to convert SJL^r mice to virus-susceptible hosts by induction of a CNS inflammatory response by pre-immunization with myelin basic protein, an encephalitogenic antigen. These studies provide valuable insights into the mechanisms of SRV susceptibility and resistance in certain inbred mice.

ROCKY MOUNTAIN OPERATIONS BRANCH
Rocky Mountain Laboratories
Hamilton, Montana
1988 Annual Report
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Annual Report
Operations Branch
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1987, to September 30, 1988

Introduction

The branch provides all services necessary to the professional staff in the pursuit of their investigations. Fiscal support includes budget management, procurement, and initiating payments and follow-up on financial obligations related to purchases, contracts, staff and official guest travel, and expenses for conferences held at RML. Other support covers the following areas: personnel, communications, library services, secretary backup service, grounds care, custodial, security, media preparation, waste disposal including hazardous wastes and radioactive wastes, glassware cleaning, photography, animal rearing and care, motor pool, operation of the power plant, and full maintenance and minor laboratory renovations in every area except electronics.

Considerable time and effort has been expended on developing and administering contracts during the past year. The new contract for operation of the glassware unit became effective on December 14, 1987.

In late October 1987 we were notified that Blue Ridge Security Guard Service, Inc., had defaulted on the guard contract at RML. Action was taken to provide security on an interim basis and to negotiate a new guard contract which became effective on July 1, 1988. The last option year for the housekeeping contract ends on September 30, 1988, and the Contracting Officer decided to not exercise the 4th Option Year on the boiler room contract. We are now in the process of negotiating contracts for housekeeping services and operation of the boiler room, which hopefully will be effective next October. We also hope to have a long-term service and maintenance contract ready to start in early October on our Beckman Instruments.

Several RMOB staff, especially Ms. Leza Hamby, have given commendable support to LPB in planning and making arrangements for the International Workshop on Bordetella pertussis to be held at RML on August 17-20, 1988.

The Maintenance Section has performed several renovation projects for LMSF, LPVD, and LPB during the past year. Most noteworthy has been the completion of a new autoclave facility and new laboratory and office space for LPVD on the third floor of Building 3, and renovation of new laboratory space for the Chlamydial Diseases Section of LMSF on the first floor of Building 4.

General Overview of the Responsibilities of Operations Branch

The fiscal and procurement department manages a budget of over \$2,000,000. Payroll is not included in this figure. It covers only the purchase of services, supplies, and equipment used in the operation of the laboratories. Timekeeping and submission of the payroll are also handled in this unit.

Personnel initiates civil service and commissioned officer personnel actions, and advises on personnel matters. This department is also charged with operation of the Job Training Partnership Act in association with the local Montana State Employment Office when the program is active. Also handled by Personnel are persons under the following programs: Stay-in-School, Summer Aides, Student Volunteers, Special Volunteers, Visiting Program, and students studying for advanced degrees.

Most of the biological media used in the research laboratories is prepared in a special laboratory by a technician.

The Graphic Arts Department provides full professional services necessary in the laboratories with the exception of medical artistry.

The library provides a full range of services for the RML staff, i.e., selections and acquisitions, cataloging, circulation, inter-library loans, reference and bibliographic services, computerized data base searches, and preparing periodicals for binding.

The Animal Unit raises guinea pigs, 25 strains of mice, eight strains of hamsters, and a colony of microtus. They are raising 113,450 animals for research from 49,566 breeding stock (approximate figures). An additional 3,000 animals are purchased annually from outside sources, including mink, rabbits, mice, and hamsters. After rearing, care is provided for these animals while they are under experiment. The most recent survey indicates an average daily holding of 30,251 small animals.

The Chief of the Branch is responsible for labor management work and administering the technical aspects of the contracts for Security, Custodial, operation of the Boiler Room and the Glassware Unit with the respective private contractors. Security is provided by a guard on duty every night and all day on weekends and holidays. Custodial services are provided in five laboratory buildings daily except weekends and holidays. Boiler Room operation provides heat, steam, compressed air, vacuum, and emergency power to the entire laboratory complex. Glassware is cleaned and sterilized in the Glassware Unit for reuse in the laboratories.

The Maintenance Department provides repair, service and renovation work in plumbing, electrical, sheet metal, carpentry, air conditioning, and refrigeration, including ultra-low temperature boxes. With the exception of electronic work, all maintenance is done by the staff. Also provided are demineralized and distilled water. A motor pool consisting of 10 vehicles is maintained. Grounds care including snow removal is provided.



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